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(54) Title: NOVEL PLANT ACYLTRANSFERASES		

(57) Abstract

By this invention, novel nucleic acid sequences encoding for acyltransferase related proteins are provided, wherein said acyltransferase-like protein is active in the transfer of a fatty acyl group from a fatty acyl donor to a fatty acyl acceptor. Also considered are amino acid and nucleic acid sequences obtainable from AT-like nucleic acid sequences and the use of such sequences to provide transgenic host cells capable of producing modified lipid content and composition.

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NOVEL PLANT ACYLTRANSFERASES

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INTRODUCTION

This application claims the benefit of U.S. Provisional Application Serial No. 60/101,939 filed September 25, 1998.

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Technical Field

The present invention is directed to nucleic acid and amino acid sequences and constructs, and methods related thereto.

15 Background

Through the development of plant genetic engineering techniques, it is now possible to produce transgenic varieties of plant species to provide plants which have novel and desirable characteristics. For example, it is now possible to genetically engineer plants for tolerance to environmental stresses, such as resistance to pathogens and tolerance to herbicides and to improve the quality characteristics of the plant, for example improved fatty acid compositions. However, the number of useful nucleotide sequences for the engineering of such characteristics is thus far limited and the speed with which new useful nucleotide sequences for engineering new characteristics is slow.

The characterization of various acyltransferase proteins is useful for the further study of plant fatty acid synthesis systems and for the development of novel and/or alternative oils sources. Studies of plant mechanisms may provide means to further enhance, control, modify, or otherwise alter the total fatty acyl composition of triglycerides and oils. Furthermore, the elucidation of the factor(s) critical to the natural production of fatty acids in plants is desired, including the purification of such factors and the characterization of element(s) and/or cofactors which enhance the efficiency of the system. Of particular interest are the nucleic acid sequences of genes encoding proteins which may be useful for applications in genetic engineering.

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SUMMARY OF THE INVENTION

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The present invention provides nucleic acid encoding for amino acid sequences for a class of proteins which are related to acyltransferase proteins. Such proteins are referred to herein as acyltransferase related or acyltransferase like proteins.

By this invention, nucleic acid sequences encoding these acyltransferase related proteins may now be characterized with respect to enzyme activity. In particular, identification and isolation of nucleic acid sequences encoding for acyltransferase related proteins from *Arabidopsis*, yeast, corn, and soybean are provided.

Thus, this invention encompasses acyltransferase related nucleic acid sequences and the corresponding amino acid sequences, and the use of these nucleic acid sequences in the preparation of oligonucleotides containing such acyltransferase related encoding sequences for analysis and recovery of plant acyltransferase related gene sequences. The acyltransferase related encoding sequence may encode a complete or partial sequence depending upon the intended use. All or a portion of the genomic sequence, or cDNA sequence, is intended.

Of special interest are recombinant DNA constructs which provide for transcription or transcription and translation (expression) of the acyltransferase related sequences in host cells. In particular, constructs which are capable of transcription or transcription and translation in plant host cells are preferred. For some applications a reduction in sequences encoding acyltransferase related sequences may be desired. Thus, recombinant constructs may be designed having the acyltransferase related sequences in a reverse orientation for expression of an anti-sense sequence or use of co-suppression, also known as "transwitch", constructs may be useful. Such constructs may contain a variety of regulatory regions including transcriptional initiation regions obtained from genes preferentially expressed in plant seed tissue. For some uses, it may be desired to use the transcriptional and translational initiation regions of the acyltransferase related gene either with the acyltransferase related encoding sequence or to direct the transcription and translation of a heterologous sequence.

Also considered in this invention are the plants and seeds containing the constructs and polynucleotides of this invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 provides the 204 amino acid conserved sequence profile identified from comparisons of glycerol-3-phosphate acyltransferase and various lysophosphatidic acid acyltransferase using PSI-BLAST.

Figure 2 provides an amino acid sequence alignment for the acyltransferase sequences. The alignment shown is of the regions of the protein extending from about 30 amino acids prior to the conserved H in the conserved sequence HXXXXD to 100 amino acids after, or downstream, of the P in the conserved PEG sequence motif of the acyltransferase-like sequences.

Figure 3 provides schematics showing the relationship of the identified acyltransferases. The relationships described are derived from an alignment of the regions of the protein extending from about 30 amino acids prior to the conserved H in the conserved sequence HXXXXD to 100 amino acids after, or downstream, of the P in the conserved PEG sequence motif of the acyltransferase-like sequences. Figure 3A provide a phylogenetic tree showing the relationship of several acyltransferases. Figure 3B provides a table showing the percent similarities and percent divergence of the novel acyltransferases and known acyltransferases using the Clustal method with PAM250 residue weight table.

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DETAILED DESCRIPTION OF THE INVENTION

In accordance with the subject invention, nucleotide sequences are provided which are capable of coding sequences of amino acids, such as, a protein, polypeptide or peptide, which are related to nucleic acid sequences encoding acyltransferase proteins, referred to herein as acyltransferase-like or acyltransferase related. The novel nucleic acid sequences find use in the preparation of constructs to direct their expression in a host cell. Furthermore, the novel nucleic acid sequences may find use in the preparation of plant expression constructs to modify the fatty acid composition of a plant cell.

In one embodiment of the present invention, nucleic acid sequences, also referred to herein as polynucleotides, are identified from databases which are related to acyltransferases.

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Isolated proteins, Polypeptides and Polynucleotides

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A first aspect of the present invention relates to isolated acyltransferase polynucleotides. The polynucleotide sequences of the present invention include isolated polynucleotides that encode the polypeptides of the invention having a deduced amino acid sequence selected from the group of sequences set forth in the Sequence Listing and to other polynucleotide sequences closely related to such sequences and variants thereof.

The invention provides a polynucleotide sequence identical over its entire length to each coding sequence as set forth in the Sequence Listing. The invention also provides the coding sequence for the mature polypeptide or a fragment thereof, as well as the coding sequence for the mature polypeptide or a fragment thereof in a reading frame with other coding sequences, such as those encoding a leader or secretory sequence, a pre-, pro-, or prepro- protein sequence. The polynucleotide can also include non-coding sequences, including for example, but not limited to, non-coding 5' and 3' sequences, such as the transcribed, untranslated sequences, termination signals, ribosome binding sites, sequences that stabilize mRNA, introns, polyadenylation signals, and additional coding sequence that encodes additional amino acids. For example, a marker sequence can be included to facilitate the purification of the fused polypeptide. Polynucleotides of the present invention also include polynucleotides comprising a structural gene and the naturally associated sequences that control gene expression.

The invention also includes polynucleotides of the formula:

$$X-(R_1)_n-(R_2)-(R_3)_n-Y$$

wherein, at the 5' end, X is hydrogen, and at the 3' end, Y is hydrogen or a metal, R₁ and R₃ are any nucleic acid residue, n is an integer between 1 and 3000, preferably between 1 and 1000 and R₂ is a nucleic acid sequence of the invention, particularly a nucleic acid sequence selected from the group set forth in the Sequence Listing and preferably SEQ IDNOs: 1, 3, 5, 7, 9, 10, 12, 14, 16, 18, 20, 22, and 226-233. In the formula, R₂ is oriented so that its 5' end residue is at the left, bound to R₁, and its 3' end residue is at the right, bound to R₃. Any stretch of nucleic acid residues denoted by either R group, where R is greater than 1, may be either a heteropolymer or a homopolymer, preferably a heteropolymer.

The invention also relates to variants of the polynucleotides described herein that encode for variants of the polypeptides of the invention. Variants that are fragments of the polynucleotides of the invention can be used to synthesize full-length polynucleotides of the

invention. Preferred embodiments are polynucleotides encoding polypeptide variants wherein 5 to 10, 1 to 5, 1 to 3, 2, 1 or no amino acid residues of a polypeptide sequence of the invention are substituted, added or deleted, in any combination. Particularly preferred are substitutions, additions, and deletions that are silent such that they do not alter the properties or activities of the polynucleotide or polypeptide.

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Nucleotide sequences encoding acyltransferases may be obtained from natural sources or be partially or wholly artificially synthesized. They may directly correspond to an acyltransferase endogenous to a natural source or contain modified amino acid sequences, such as sequences which have been mutated, truncated, increased or the like. Acyltransferases may be obtained by a variety of methods, including but not limited to, partial or homogenous purification of protein extracts, protein modeling, nucleic acid probes, antibody preparations and sequence comparisons. Typically an acyltransferase will be derived in whole or in part from a natural source. A natural source includes, but is not limited to, prokaryotic and eukaryotic sources, including, bacteria, yeasts, plants, including algae, and the like.

Of special interest are acyltransferases which are obtainable from eukaryotic sources, including those which are obtained, from plants, or from acyltransferases which are obtainable through the use of these sequences. "Obtainable" refers to those acyltransferases which have sufficiently similar sequences to that of the sequences provided herein to provide a biologically active protein of the present invention.

Further preferred embodiments of the invention that are at least 50%, 60%, or 70% identical over their entire length to a polynucleotide encoding a polypeptide of the invention, and polynucleotides that are complementary to such polynucleotides. More preferable are polynucleotides that comprise a region that is at least 80% identical over its entire length to a polynucleotide encoding a polypeptide of the invention and polynucleotides that are complementary thereto. In this regard, polynucleotides at least 90% identical over their entire length are particularly preferred, those at least 95% identical are especially preferred. Further, those with at least 97% identity are highly preferred and those with at least 98% and 99% identity are particularly highly preferred, with those at least 99% being the most highly preferred.

Preferred embodiments are polynucleotides that encode polypeptides that retain substantially the same biological function or activity as the mature polypeptides encoded by the polynucleotides set forth in the Sequence Listing.

The invention further relates to polynucleotides that hybridize to the above-described sequences. In particular, the invention relates to polynucleotides that hybridize under stringent conditions to the above-described polynucleotides. As used herein, the terms "stringent conditions" and "stringent hybridization conditions" mean that hybridization will generally occur if there is at least 95% and preferably at least 97% identity between the sequences. An example of stringent hybridization conditions is overnight incubation at 42°C in a solution comprising 50% formamide, 5x SSC (150 mM NaCl, 15 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 micrograms/milliliter denatured, sheared salmon sperm DNA, followed by washing the hybridization support in 0.1x SSC at approximately 65°C. Other hybridization and wash conditions are well known and are exemplified in Sambrook, et al., Molecular Cloning: A Laboratory Manual, Second Edition, cold Spring Harbor, NY (1989), particularly Chapter 11.

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The invention also provides a polynucleotide consisting essentially of a polynucleotide sequence obtainable by screening an appropriate library containing the complete gene for a polynucleotide sequence set for in the Sequence Listing under stringent hybridization conditions with a probe having the sequence of said polynucleotide sequence or a fragment thereof; and isolating said polynucleotide sequence. Fragments useful for obtaining such a polynucleotide include, for example, probes and primers as described herein.

As discussed herein regarding polynucleotide assays of the invention, for example, polynucleotides of the invention can be used as a hybridization probe for RNA, cDNA, or genomic DNA to isolate full length cDNAs or genomic clones encoding a polypeptide and to isolate cDNA or genomic clones of other genes that have a high sequence similarity to a polynucleotide set forth in the Sequence Listing. Such probes will generally comprise at least 15 bases. Preferably such probes will have at least 30 bases and can have at least 50 bases. Particularly preferred probes will have between 30 bases and 50 bases, inclusive.

The coding region of each gene that comprises or is comprised by a polynucleotide sequence set forth in the Sequence Listing may be isolated by screening using a DNA sequence provided in the Sequence Listing to synthesize an oligonucleotide probe. A labeled oligonucleotide having a sequence complementary to that of a gene of the invention is then used to screen a library of cDNA, genomic DNA or mRNA to identify members of the library which hybridize to the probe. For example, synthetic oligonucleotides are prepared which correspond to the N-terminal sequence of the polypeptide. The partial sequences so prepared can then be used as probes to obtain acyltransferase clones from a gene library prepared from

a cell source of interest. Alternatively, where oligonucleotides of low degeneracy can be prepared from particular peptides, such probes may be used directly to screen gene libraries for gene sequences. In particular, screening of cDNA libraries in phage vectors is useful in such methods due to lower levels of background hybridization.

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Typically, a sequence obtainable from the use of nucleic acid probes will show 60-70% sequence identity between the target acyltransferase sequence and the encoding sequence used as a probe. However, lengthy sequences with as little as 50-60% sequence identity may also be obtained. The nucleic acid probes may be a lengthy fragment of the nucleic acid sequence, or may also be a shorter, oligonucleotide probe. When longer nucleic acid fragments are employed as probes (greater than about 100 bp), one may screen at lower stringencies in order to obtain sequences from the target sample which have 20-50% deviation (i.e., 50-80% sequence homology) from the sequences used as probe. Oligonucleotide probes can be considerably shorter than the entire nucleic acid sequence encoding an acyltransferase enzyme, but should be at least about 10, preferably at least about 15, and more preferably at least about 20 nucleotides. A higher degree of sequence identity is desired when shorter regions are used as opposed to longer regions. It may thus be desirable to identify regions of highly conserved amino acid sequence to design oligonucleotide probes for detecting and recovering other related genes. Shorter probes are often particularly useful for polymerase chain reactions (PCR), especially when highly conserved sequences can be identified. (See, Gould, et al., PNAS USA (1989) 86:1934-1938).

The skilled artisan will appreciate that, in many cases, an isolated cDNA sequence will be incomplete, in that the region coding for the polypeptide is truncated with respect to the 5' terminus of the cDNA. This is a consequence of the reverse transcriptase, an enzyme with low 'processivity' (a measure of the ability of the enzyme to remain attached to the template during the polymerization reaction) employed during the first strand cDNA synthesis.

There are several methods available and are well know to the skilled artisan to obtain full-length cDNAs, or extend short cDNAs, for example those based on the method of Rapid Amplification of cDNA Ends (RACE) (see, for example, Frohman *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:8998-9002). Recent modifications of the technique, exemplified by the Marathon™ technology (Clonetech Laboratories, Inc.) for example, have significantly simplified obtaining full-length cDNA sequences.

Another aspect of the present invention relates to isolated acyltransferase polypeptides. Such polypeptides include isolated polypeptides set forth in the Sequence Listing, as well as polypeptides and fragments thereof, particularly those polypeptides which exhibit acyltransferase activity and also those polypeptides which have at least 50%, 60% or 70% identity, preferably at least 80% identity, more preferably at least 90% identity, and most preferably at least 95% identity to a polypeptide sequence selected from the group of sequences set forth in the Sequence Listing, and also include portions of such polypeptides, wherein such portion of the polypeptide preferably includes at least 30 amino acids and more preferably includes at least 50 amino acids.

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"Identity", as is well understood in the art, is a relationship between two or more polypeptide sequences or two or more polynucleotide sequences, as determined by comparing the sequences. In the art, "identity" also means the degree of sequence relatedness between polypeptide or polynucleotide sequences, as determined by the match between strings of such sequences. "Identity" can be readily calculated by known methods including, but not limited to, those described in Computational Molecular Biology, Lesk, A.M., ed., Oxford University Press, New York (1988); Biocomputing: Informatics and Genome Projects, Smith, D.W., ed., Academic Press, New York, 1993; Computer Analysis of Sequence Data, Part I, Griffin, A.M. and Griffin, H.G., eds., Humana Press, New Jersey (1994); Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press (1987); Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., Stockton Press, New York (1991); and Carillo, H., and Lipman, D., SIAM J Applied Math, 48:1073 (1988). Methods to determine identity are designed to give the largest match between the sequences tested. Moreover, methods to determine identity are codified in publicly available programs. Computer programs which can be used to determine identity between two sequences include, but are not limited to, GCG (Devereux, J., et al., Nucleic Acids Research 12(1):387 (1984); suite of five BLAST programs, three designed for nucleotide sequences queries (BLASTN, BLASTX, and TBLASTX) and two designed for protein sequence queries (BLASTP and TBLASTN) (Coulson, Trends in Biotechnology, 12: 76-80 (1994); Birren, et al., Genome Analysis, 1: 543-559 (1997)). The BLAST X program is publicly available from NCBI and other sources (BLAST Manual, Altschul, S., et al., NCBI NLM NIH, Bethesda, MD 20894; Altschul, S., et 30 al., J. Mol. Biol., 215:403-410 (1990)). The well known Smith Waterman algorithm can also be used to determine identity.

Parameters for polypeptide sequence comparison typically include the following:

Algorithm: Needleman and Wunsch, J. Mol. Biol. 48:443-453 (1970)

Comparison matrix: BLOSSUM62 from Hentikoff and Hentikoff, *Proc. Natl. Acad.*Sci USA 89:10915-10919 (1992)

Gap Penalty: 12

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Gap Length Penalty: 4

A program which can be used with these parameters is publicly available as the "gap" program from Genetics Computer Group, Madison Wisconsin. The above parameters along with no penalty for end gap are the default parameters for peptide comparisons.

Parameters for polynucleotide sequence comparison include the following:

Algorithm: Needleman and Wunsch, J. Mol. Biol. 48:443-453 (1970)

Comparison matrix: matches = +10; mismatches = 0

Gap Penalty: 50

Gap Length Penalty: 3

A program which can be used with these parameters is publicly available as the "gap" program from Genetics Computer Group, Madison Wisconsin. The above parameters are the default parameters for nucleic acid comparisons.

The invention also includes polypeptides of the formula:

$$X-(R_1)_{n}-(R_2)-(R_3)_{n}-Y$$

wherein, at the amino terminus, X is hydrogen, and at the carboxyl terminus, Y is hydrogen or a metal, R_1 and R_3 are any amino acid residue, n is an integer between 1 and 1000, and R_2 is an amino acid sequence of the invention, particularly an amino acid sequence selected from the group set forth in the Sequence Listing and preferably SEQ IDNOs: 2, 4, 6, 8, 11, 13, 15, 17, 19, 21, 23, and 218-225. In the formula, R_2 is oriented so that its amino terminal residue is at the left, bound to R_1 , and its carboxy terminal residue is at the right, bound to R_3 . Any stretch of amino acid residues denoted by either R group, where R is greater than 1, may be either a heteropolymer or a homopolymer, preferably a heteropolymer.

Polypeptides of the present invention include isolated polypeptides encoded by a polynucleotide comprising a sequence selected from the group of a sequence contained in SEQ ID NOs: 1, 3, 5, 7, 9, 10, 12, 14, 16, 18, 20, 22, and 226-233.

The polypeptides of the present invention can be mature protein or can be part of a fusion protein.

Fragments and variants of the polypeptides are also considered to be a part of the invention. A fragment is a variant polypeptide which has an amino acid sequence that is entirely the same as part but not all of the amino acid sequence of the previously described polypeptides. The fragments can be "free-standing" or comprised within a larger polypeptide of which the fragment forms a part or a region, most preferably as a single continuous region. Preferred fragments are biologically active fragments which are those fragments that mediate activities of the polypeptides of the invention, including those with similar activity or improved activity or with a decreased activity. Also included are those fragments that antigenic or immunogenic in an animal, particularly a human.

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Variants of the polypeptide also include polypeptides that vary from the sequences set forth in the Sequence Listing by conservative amino acid substitutions, substitution of a residue by another with like characteristics. In general, such substitutions are among Ala, Val, Leu and Ile; between Ser and Thr; between Asp and Glu; between Asp and Gln; between Lys and Arg; or between Phe and Tyr. Particularly preferred are variants in which 5 to 10; 1 to 5; 1 to 3 or one amino acid(s) are substituted, deleted, or added, in any combination.

Variants that are fragments of the polypeptides of the invention can be used to produce the corresponding full length polypeptide by peptide synthesis. Therefore, these variants can be used as intermediates for producing the full-length polypeptides of the invention.

The polynucleotides and polypeptides of the invention can be used, for example, in the transformation of various host cells, as further discussed herein.

The invention also provides polynucleotides that encode a polypeptide that is a mature protein plus additional amino or carboxyl-terminal amino acids, or amino acids within the mature polypeptide (for example, when the mature form of the protein has more than one polypeptide chain). Such sequences can, for example, play a role in the processing of a protein from a precursor to a mature form, allow protein transport, shorten or lengthen protein half-life, or facilitate manipulation of the protein in assays or production. It is contemplated that cellular enzymes can be used to remove any additional amino acids from the mature protein.

A precursor protein, having the mature form of the polypeptide fused to one or more prosequences may be an inactive form of the polypeptide. The inactive precursors generally are activated when the prosequences are removed. Some or all of the prosequences may be removed prior to activation. Such precursor protein are generally called proproteins.

The polynucleotide and polypeptide sequences can also be used to identify additional sequences which are homologous to the sequences of the present invention. The most preferable and convenient method is to store the sequence in a computer readable medium, for example, floppy disk, CD ROM, hard disk drives, external disk drives and DVD, and then to use the stored sequence to search a sequence database with well known searching tools. 5 Examples of public databases include the DNA Database of Japan (DDBJ)(http://www.ddbj.nig.ac.jp/); Genebank (http://www.ncbi.nlm.nih.gov/web/Genbank/Index.html); and the European Molecular Biology Laboratory Nucleic Acid Sequence Database (EMBL) (http://www.ebi.ac.uk/ebi_docs/embl_db.html). A number of different search algorithms are 10 available to the skilled artisan, one example of which are the suite of programs referred to as BLAST programs. There are five implementations of BLAST, three designed for nucleotide sequences queries (BLASTN, BLASTX, and TBLASTX) and two designed for protein sequence queries (BLASTP and TBLASTN) (Coulson, Trends in Biotechnology, 12: 76-80 (1994); Birren, et al., Genome Analysis, 1: 543-559 (1997)). Additional programs are 15 available in the art for the analysis of identified sequences, such as sequence alignment

Plant Constructs and Methods of Use

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and are well known to the skilled artisan.

Of interest in the present invention, is the use of the nucleotide sequences, or polynucleotides, in recombinant DNA constructs to direct the transcription or transcription and translation (expression) of the acyltransferase sequences of the present invention in a host cell.

programs, programs for the identification of more distantly related sequences, and the like,

Of particular interest is the use of the nucleotide sequences, or polynucleotides, in recombinant DNA constructs to direct the transcription or transcription and translation (expression) of the acyltransferase sequences of the present invention in a host cell. The expression constructs generally comprise a promoter functional in a host cell operably linked to a nucleic acid sequence encoding an acyltransferase of the present invention and a transcriptional termination region functional in a host cell.

By "host cell" is meant a cell which contains a vector and supports the replication, and/or transcription or transcription and translation (expression) of the expression construct.

Host cells for use in the present invention can be prokaryotic cells, such as *E. coli*, or eukaryotic cells such as yeast, plant, insect, amphibian, or mammalian cells. Preferably, host cells are monocotyledenous or dicotyledenous plant cells.

Of particular interest in the present invention is the use of the polynucleotides of the present invention for the preparation of constructs to direct the transcription or transcription and translation of the nucleotide sequences encoding an acyltransferase in a host plant cell. Plant expression constructs generally comprise a promoter functional in a plant host cell operably linked to a nucleic acid sequence of the present and a transcriptional termination region functional in a host plant cell.

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Those skilled in the art will recognize that there are a number of promoters which are functional in plant cells, and have been described in the literature. Chloroplast and plastid specific promoters, chloroplast or plastid functional promoters, and chloroplast or plastid operable promoters are also envisioned.

One set of promoters are constitutive promoters such as the CaMV35S or FMV35S promoters that yield high levels of expression in most plant organs. Enhanced or duplicated versions of the CaMV35S and FMV35S promoters are useful in the practice of this invention (Odell, et al. (1985) Nature 313:810-812; Rogers, U.S. Patent Number 5,378, 619). In addition, it may also be preferred to bring about expression of the protein of interest in specific tissues of the plant, such as leaf, stem, root, tuber, seed, fruit, etc., and the promoter chosen should have the desired tissue and developmental specificity.

Of particular interest is the expression of the nucleic acid sequences of the present invention from transcription initiation regions which are preferentially expressed in a plant seed tissue. Examples of such seed preferential transcription initiation sequences include those sequences derived from sequences encoding plant storage protein genes or from genes involved in fatty acid biosynthesis in oilseeds. Examples of such promoters include the 5' regulatory regions from such genes as napin (Kridl *et al.*, *Seed Sci. Res. 1:*209:219 (1991)), phaseolin, zein, soybean trypsin inhibitor, ACP, stearoyl-ACP desaturase, soybean α' subunit of β-conglycinin (soy 7s, (Chen *et al.*, *Proc. Natl. Acad. Sci.*, 83:8560-8564 (1986))) and oleosin.

It may be advantageous to direct the localization of proteins conferring acyltransferase to a particular subcellular compartment, for example, to the mitochondrion, endoplasmic reticulum, vacuoles, chloroplast or other plastidic compartment. For example, where the genes of interest of the present invention will be targeted to plastids, such as chloroplasts, for

expression, the constructs will also employ the use of sequences to direct the gene to the plastid. Such sequences are referred to herein as chloroplast transit peptides (CTP) or plastid transit peptides (PTP). In this manner, where the gene of interest is not directly inserted into the plastid, the expression construct will additionally contain a gene encoding a transit peptide to direct the gene of interest to the plastid. The chloroplast transit peptides may be derived from the gene of interest, or may be derived from a heterologous sequence having a CTP. Such transit peptides are known in the art. See, for example, Von Heijne et al. (1991) Plant Mol. Biol. Rep. 9:104-126; Clark et al. (1989) J. Biol. Chem. 264:17544-17550; della-Cioppa et al. (1987) Plant Physiol. 84:965-968; Romer et al. (1993) Biochem. Biophys. Res Commun. 196:1414-1421; and, Shah et al. (1986) Science 233:478-481. Additional transit peptides for the translocation of the protein to the endoplasmic reticulum (ER), or vacuole may also find use in the constructs of the present invention.

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Depending upon the intended use, the constructs may contain the nucleic acid sequence which encodes the entire acyltransferase protein, or a portion thereof. For example, where antisense inhibition of a given acyltransferase protein is desired, the entire sequence is not required. Furthermore, where acyltransferase sequences used in constructs are intended for use as probes, it may be advantageous to prepare constructs containing only a particular portion of a acyltransferase encoding sequence, for example a sequence which is discovered to encode a highly conserved acyltransferase region.

The skilled artisan will recognize that there are various methods for the inhibition of expression of endogenous sequences in a host cell. Such methods include, but are not limited to antisense suppression (Smith, et al. (1988) Nature 334:724-726), co-suppression (Napoli, et al. (1989) Plant Cell 2:279-289), ribozymes (PCT Publication WO 97/10328), and combinations of sense and antisense, such as those described by Waterhouse, et al. (1998) Proc. Natl. Acad. Sci. USA 95:13959-13964. Methods for the suppression of endogenous sequences in a host cell typically employ the transcription or transcription and translation of at least a portion of the sequence to be suppressed. Such sequences may be homologous to coding as well as non-coding regions of the endogenous sequence.

Regulatory transcript termination regions may be provided in plant expression constructs of this invention as well. Transcript termination regions may be provided by the DNA sequence encoding the acyltransferase or a convenient transcription termination region derived from a different gene source, for example, the transcript termination region which is naturally associated with the transcript initiation region. The skilled artisan will recognize

that any convenient transcript termination region which is capable of terminating transcription in a plant cell may be employed in the constructs of the present invention.

Alternatively, constructs may be prepared to direct the expression of the acyltransferase sequences directly from the host plant cell plastid. Such constructs and methods are known in the art and are generally described, for example, in Svab, et al. (1990) Proc. Natl. Acad. Sci. USA 87:8526-8530 and Svab and Maliga (1993) Proc. Natl. Acad. Sci. USA 90:913-917 and in U.S. Patent Number 5,693,507.

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A plant cell, tissue, organ, or plant into which the recombinant DNA constructs containing the expression constructs have been introduced is considered transformed, transfected, or transgenic. A transgenic or transformed cell or plant also includes progeny of the cell or plant and progeny produced from a breeding program employing such a transgenic plant as a parent in a cross and exhibiting an altered genotype resulting from the presence of an introduced acyltransferase nucleic acid sequence.

The term "introduced" in the context of inserting a nucleic acid sequence into a cell, means "transfection", or "transformation" or "transduction" and includes reference to the incorporation of a nucleic acid sequence into a eukaryotic or prokaryotic cell where the nucleic acid sequence may be incorporated into the genome of the cell (for example, chromosome, plasmid, plastid, or mitochondrial DNA), converted into an autonomous replicon, or transiently expressed (for example, transfected mRNA).

Plant expression or transcription constructs having an acyltransferase as the DNA sequence of interest for increased or decreased expression thereof may be employed with a wide variety of plant life, particularly, plant life involved in the production of vegetable oils for edible and industrial uses. Plants of interest in the present invention include monocotyledenous and dicotyledenous plants. Most especially preferred are temperate oilseed crops. Plants of interest include, but are not limited to, rapeseed (Canola and High Erucic Acid varieties), sunflower, safflower, cotton, soybean, peanut, coconut and oil palms, and corn. Depending on the method for introducing the recombinant constructs into the host cell, other DNA sequences may be required. Importantly, this invention is applicable to dicotyledyons and monocotyledons species alike and will be readily applicable to new and/or improved transformation and regulation techniques.

As used herein, the term "plant" includes reference to whole plants, plant organs (for example, leaves, stems, roots, etc.), seeds, and plant cells and progeny of same. Plant cell, as used herein includes, without limitation, seeds suspension cultures, embryos, meristematic

regions, callus tissue, leaves roots shoots, gametophytes, sporophytes, pollen, and microspores. The class of plants which can be used in the methods of the present invention is generally as broad as the class of higher plants amenable to transformation techniques, including both monocotyledenous and dicotyledenous plants. Particularly preferred plants of interest include, but are not limited to, rapeseed (Canola and High Erucic Acid varieties), sunflower, safflower, cotton, soybean, peanut, coconut and oil palms, and corn. Most especially preferred plants include *Brassica*, soybean, and corn.

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As used herein, "transgenic plant" includes reference to a plant which comprises within its genome a heterologous polynucleotide. Generally, the heterologous polynucleotide is stably integrated within the genome such that the polynucleotide is passed on to successive generations. The heterologous polynucleotide may be integrated into the genome alone or as part of a recombinant expression cassette. "Transgenic" is used herein to include any cell, cell line, callus, tissue, plant part or plant, the genotype of which has been altered by the presence of heterologous nucleic acid including those transgenics initially so altered as well as those created by sexual crosses or asexual propagation from the initial transgenic.

Thus a plant having within its cells a heterologous polynucleotide is referred to herein as a transgenic plant. The heterologous polynucleotide can be either stably integrated into the genome, or can be extra-chromosomal. Preferably, the polynucleotide of the present invention is stably integrated into the genome such that the polynucleotide is passed on to successive generations. The polynucleotide is integrated into the genome alone or as part of a recombinant expression cassette. "Transgenic" is used herein to include any cell, cell line, callus, tissue, plant part or plant, the genotype of which has been altered by the presence of heterologous nucleic acids including those transgenics initially so altered as well as those created by sexual crosses or asexual reproduction of the initial transgenics.

As used herein, "heterologous" in reference to a nucleic acid is a nucleic acid that originates from a foreign species, or, if from the same species, is substantially modified from its native form in composition and/or genomic locus by deliberate human intervention. For example, a promoter operably linked to a heterologous structural gene is from a species different from that from which the structural gene was derived, or, if from the same species, one or both are substantially modified from their original form. A heterologous protein may originate from a foreign species, or, if from the same species, is substantially modified from its original form by deliberate human intervention.

As used herein, a "recombinant expression cassette" is a nucleic acid construct, generated recombinantly or synthetically, with a series of specified nucleic acid elements which permit transcription of a particular nucleic acid in a target cell. The recombinant expression cassette can be incorporated into a plasmid, chromosome, mitochondrial DNA, plastid DNA, virus, or nucleic acid fragment. Typically, the recombinant expression cassette portion of an expression vector includes, among other sequences, a nucleic acid sequence to be transcribed and a promoter.

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It is contemplated that the gene sequences may be synthesized, either completely or in part, especially where it is desirable to provide plant-preferred sequences. Thus, all or a portion of the desired structural gene (that portion of the gene which encodes the acyltransferase protein) may be synthesized using codons preferred by a selected host. Host-preferred codons may be determined, for example, from the codons used most frequently in the proteins expressed in a desired host species.

One skilled in the art will readily recognize that antibody preparations, nucleic acid probes (DNA and RNA) and the like may be prepared and used to screen and recover "homologous" or "related" acyltransferase from a variety of plant sources. Homologous sequences are found when there is an identity of sequence, which may be determined upon comparison of sequence information, nucleic acid or amino acid, or through hybridization reactions between a known acyltransferase and a candidate source. Conservative changes, such as Glu/Asp, Val/Ile, Ser/Thr, Arg/Lys and Gln/Asn may also be considered in determining sequence homology. Amino acid sequences are considered homologous by as little as 25% sequence identity between the two complete mature proteins. (See generally, Doolittle, R.F., OF URFS and ORFS (University Science Books, CA, 1986.)

Thus, other acyltransferase sequences can be obtained from the specific exemplified sequences provided herein. Furthermore, it will be apparent that one can obtain natural and synthetic sequences, including modified amino acid sequences and starting materials for synthetic-protein modeling from the exemplified sequences and from acyltransferases which are obtained through the use of such exemplified sequences. Modified amino acid sequences include sequences which have been mutated, truncated, increased and the like, whether such sequences were partially or wholly synthesized. Sequences which are actually purified from plant preparations or are identical or encode identical proteins thereto, regardless of the method used to obtain the protein or sequence, are equally considered naturally derived.

For immunological screening, antibodies to the acyltransferase protein can be prepared by injecting rabbits or mice with the purified protein or portion thereof, such methods of preparing antibodies being well known to those in the art. Either monoclonal or polyclonal antibodies can be produced, although typically polyclonal antibodies are more useful for gene isolation. Western analysis may be conducted to determine that a related protein is present in a crude extract of the desired plant species, as determined by cross-reaction with the antibodies to the acyltransferase protein. When cross-reactivity is observed, genes encoding the related proteins are isolated by screening expression libraries representing the desired plant species. Expression libraries can be constructed in a variety of commercially available vectors, including lambda gt11, as described in Sambrook, et al. (Molecular Cloning: A Laboratory Manual, Second Edition (1989) Cold Spring Harbor Laboratory, Cold Spring Harbor, New York).

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The nucleic acid sequences associated with acyltransferase proteins will find many uses. For example, recombinant constructs can be prepared which can be used as probes, or which will provide for expression of the acyltransferase protein in host cells to produce a ready source of the enzyme and/or to modify the composition of triglycerides found therein. Other useful applications may be found when the host cell is a plant host cell, either *in vitro* or *in vivo*.

The modification of fatty acid compositions may also affect the fluidity of plant membranes. Different lipid concentrations have been observed in cold-hardened plants, for example. By this invention, one may be capable of introducing traits which will lend to chill tolerance. Constitutive or temperature inducible transcription initiation regulatory control regions may have special applications for such uses.

As discussed above, nucleic acid sequence encoding an acyltransferase of this invention may include genomic, cDNA or mRNA sequence. By "encoding" is meant that the sequence corresponds to a particular amino acid sequence either in a sense or anti-sense orientation. By "extrachromosomal" is meant that the sequence is outside of the plant genome of which it is naturally associated. By "recombinant" is meant that the sequence contains a genetically engineered modification through manipulation via mutagenesis, restriction enzymes, and the like.

Once the desired acyltransferase nucleic acid sequence is obtained, it may be manipulated in a variety of ways. Where the sequence involves non-coding flanking regions, the flanking regions may be subjected to resection, mutagenesis, etc. Thus, transitions,

transversions, deletions, and insertions may be performed on the naturally occurring sequence. In addition, all or part of the sequence may be synthesized. In the structural gene, one or more codons may be modified to provide for a modified amino acid sequence, or one or more codon mutations may be introduced to provide for a convenient restriction site or other purpose involved with construction or expression. The structural gene may be further modified by employing synthetic adapters, linkers to introduce one or more convenient restriction sites, or the like.

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The nucleic acid or amino acid sequences encoding an acyltransferase of this invention may be combined with other non-native, or "heterologous", sequences in a variety of ways. By "heterologous" sequences is meant any sequence which is not naturally found joined to the acyltransferase, including, for example, combinations of nucleic acid sequences from the same plant which are not naturally found joined together.

The DNA sequence encoding an acyltransferase of this invention may be employed in conjunction with all or part of the gene sequences normally associated with the acyltransferase. In its component parts, a DNA sequence encoding acyltransferase is combined in a DNA construct having, in the 5' to 3' direction of transcription, a transcription initiation control region capable of promoting transcription and translation in a host cell, the DNA sequence encoding plant acyltransferase and a transcription and translation termination region.

Potential host cells include both prokaryotic cells, such as *E.coli* and eukaryotic cells such as yeast, insect, amphibian, or mammalian cells. A host cell may be unicellular or found in a multicellular differentiated or undifferentiated organism depending upon the intended use. Preferably, host cells of the present invention include plant cells, both monocotyledenous and dicotyledenous. Cells of this invention may be distinguished by having a sequence foreign to the wild-type cell present therein, for example, by having a recombinant nucleic acid construct encoding an acyltransferase therein.

The methods used for the transformation of the host plant cell are not critical to the present invention. The transformation of the plant is preferably permanent, i.e. by integration of the introduced expression constructs into the host plant genome, so that the introduced constructs are passed onto successive plant generations. The skilled artisan will recognize that a wide variety of transformation techniques exist in the art, and new techniques are continually becoming available. Any technique that is suitable for the target host plant can be employed within the scope of the present invention. For example, the constructs can be

introduced in a variety of forms including, but not limited to as a strand of DNA, in a plasmid, or in an artificial chromosome. The introduction of the constructs into the target plant cells can be accomplished by a variety of techniques, including, but not limited to calcium-phosphate-DNA co-precipitation, electroporation, microinjection, Agrobacterium infection, liposomes or microprojectile transformation. The skilled artisan can refer to the literature for details and select suitable techniques for use in the methods of the present invention.

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Normally, included with the DNA construct will be a structural gene having the necessary regulatory regions for expression in a host and providing for selection of transformant cells. The gene may provide for resistance to a cytotoxic agent, e.g. antibiotic, heavy metal, toxin, etc., complementation providing prototrophy to an auxotrophic host, viral immunity or the like. Depending upon the number of different host species the expression construct or components thereof are introduced, one or more markers may be employed, where different conditions for selection are used for the different hosts.

Where Agrobacterium is used for plant cell transformation, a vector may be used which may be introduced into the Agrobacterium host for homologous recombination with T-DNA or the Ti- or Ri-plasmid present in the Agrobacterium host. The Ti- or Ri-plasmid containing the T-DNA for recombination may be armed (capable of causing gall formation) or disarmed (incapable of causing gall formation), the latter being permissible, so long as the vir genes are present in the transformed Agrobacterium host. The armed plasmid can give a mixture of normal plant cells and gall.

In some instances where Agrobacterium is used as the vehicle for transforming host plant cells, the expression or transcription construct bordered by the T-DNA border region(s) will be inserted into a broad host range vector capable of replication in E. coli and Agrobacterium, there being broad host range vectors described in the literature. Commonly used is pRK2 or derivatives thereof. See, for example, Ditta, et al., (Proc. Nat. Acad. Sci., U.S.A. (1980) 77:7347-7351) and EPA 0 120 515, which are incorporated herein by reference. Alternatively, one may insert the sequences to be expressed in plant cells into a vector containing separate replication sequences, one of which stabilizes the vector in E. coli, and the other in Agrobacterium. See, for example, McBride and Summerfelt (Plant Mol. Biol. (1990) 14:269-276), wherein the pRiHRI (Jouanin, et al., Mol. Gen. Genet. (1985) 201:370-374) origin of replication is utilized and provides for added stability of the plant expression vectors in host Agrobacterium cells.

Included with the expression construct and the T-DNA will be one or more markers, which allow for selection of transformed Agrobacterium and transformed plant cells. A number of markers have been developed for use with plant cells, such as resistance to chloramphenicol, kanamycin, the aminoglycoside G418, hygromycin, or the like. The particular marker employed is not essential to this invention, one or another marker being preferred depending on the particular host and the manner of construction.

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For transformation of plant cells using Agrobacterium, explants may be combined and incubated with the transformed Agrobacterium for sufficient time for transformation, the bacteria killed, and the plant cells cultured in an appropriate selective medium. Once callus forms, shoot formation can be encouraged by employing the appropriate plant hormones in accordance with known methods and the shoots transferred to rooting medium for regeneration of plants. The plants may then be grown to seed and the seed used to establish repetitive generations and for isolation of vegetable oils.

There are several possible ways to obtain the plant cells of this invention which contain multiple expression constructs. Any means for producing a plant comprising a construct having a nucleic acid sequence of the present invention, and at least one other construct having another DNA sequence encoding an enzyme are encompassed by the present invention. For example, the expression construct can be used to transform a plant at the same time as the second construct either by inclusion of both expression constructs in a single transformation vector or by using separate vectors, each of which express desired genes. The second construct can be introduced into a plant which has already been transformed with the first expression construct, or alternatively, transformed plants, one having the first construct and one having the second construct, can be crossed to bring the constructs together in the same plant.

In general, acyltransferase proteins are active in the transfer of acyl groups from a donor to a variety of different substrates. For example, diacylglycerol acyltransferases add acyl groups to diacylglycerol to form triacylglycerol (TAG), oracyl:CoA:cholesterol acyltransferase uses an acyl-CoA as a donor to transfer an acyl group to a sterol to form a sterol ester. Typically, the substrates include, but are not limited to glycerides, including mono and diglycerides, sterols, stanols, phosphatides, and the like. Donors include, but are not limited to acyl-CoA and acyl-ACP molecules.

The invention now being generally described, it will be more readily understood by reference to the following examples which are included for purposes of illustration only and are not intended to limit the present invention.

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EXAMPLES

Example 1: RNA Isolations

Total RNA from the inflorescence and developing seeds of *Arabidopsis thaliana* is isolated for use in construction of complementary (cDNA) libraries. The procedure is an adaptation of the DNA isolation protocol of Webb and Knapp (D.M. Webb and S.J. Knapp, (1990) Plant Molec. Reporter, 8, 180-185). The following description assumes the use of 1g fresh weight of tissue. Frozen seed tissue is powdered by grinding under liquid nitrogen. The powder is added to 10ml REC buffer (50mM Tris-HCl, pH 9, 0.8M NaCl, 10mM EDTA, 0.5% w/v CTAB (cetyltrimethyl-ammonium bromide)) along with 0.2g insoluble polyvinylpolypyrrolidone, and ground at room temperature. The homogenate is centrifuged for 5 minutes at 12,000 xg to pellet insoluble material. The resulting supernatant fraction is extracted with chloroform, and the top phase is recovered.

The RNA is then precipitated by addition of 1 volume RecP (50mM Tris-HCL pH9, 10mM EDTA and 0.5% (w/v) CTAB) and collected by brief centrifugation as before. The RNA pellet is redissolved in 0.4 ml of 1M NaCl. The RNA pellet is redissolved in water and extracted with phenol/chloroform. Sufficient 3M potassium acetate (pH 5) is added to make the mixture 0.3M in acetate, followed by addition of two volumes of ethanol to precipitate the RNA. After washing with ethanol, this final RNA precipitate is dissolved in water and stored frozen.

Alternatively, total RNA may be obtained using TRIzol reagent (BRL-Lifetechnologies, Gaithersburg, MD) following the manufacturers protocol. The RNA precipitate is dissolved in water and stored frozen.

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Example 2: Identification of Acyltransferase Homology Sequences

Searches are performed on a Silicon Graphics Unix computer using additional Bioaccellerator hardware and GenWeb software supplied by Compugen Ltd. This software and hardware enables the use of the Smith-Waterman algorithm in searching DNA and protein databases using profiles as queries. The program used to query protein databases is profilesearch. This is a search where the query is not a single sequence but a profile based on a multiple alignment of amino acid or nucleic acid sequences. The profile is used to query a sequence data set, i.e., a sequence database. The profile contains all the pertinent information for scoring each position in a sequence, in effect replacing the "scoring matrix" used for the standard query searches. The program used to query nucleotide databases with a protein profile is tprofilesearch. Tprofilesearch searches nucleic acid databases using an amino acid profile query. As the search is running, sequences in the database are translated to amino acid sequences in six reading frames. The output file for tprofilesearch is identical to the output file for profilesearch except for an additional column that indicates the frame in which the best alignment occurred.

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The Smith-Waterman algorithm, (Smith and Waterman (1981) *supra*), is used to search for similarities between one sequence from the query and a group of sequences contained in the database. E score values as well as other sequence information, such as conserved peptide sequences of HXXXXD and PEG are used to identify related sequences. By using the conserved peptide sequence information, E score values of greater than E-12 and E-8 are considered. For example, the EST sequence originally used to identify ATAT2 had an E score of 0.0094, while the EST sequence originally used to identify ATLPAAT1 had an E score of 0.0868.

A protein sequence of glycerol-3-phosphate from *E. coli* (Swiss Prot Accession P00482) is used to search the NCBI non-redundant protein database using BLAST. In the first round of searches, other membrane forms of G3PAAT are identified. In subsequent PSI-BLAST searches (Altschul, *et al.* (1997) *Nucleic Acids Res* 25:3389-3402), LPAATs and other acyltransferases are identified. Using sequence alignment software programs, G3PAAT and different LPAAT amino acid sequences are aligned, and a profile is generated using a homologous sequence region, between amino acids 256 and 459 of the *E. coli* sequence.

The identified 204 amino acid is used to query the protein database using PSI-BLAST. After 5 iterations of PSI-BLAST, the profile generated from this new query (Figure 1)

identified soluble forms of G3PAAT. Prior to this identification, no sequence homology had been identified between the membrane and soluble forms of G3PAAT.

Example 3: Excision of PSI-BLAST Profile

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The profile generated from the queries using PSI-BLAST is excised from the hyper text markup language (html) file. The worldwide web (www)/html interface to psiblast at ncbi stores the current generated profile matrix in a hidden field in the html file that is returned after each iteration of psiblast. However, this matrix has been encoded into string62 (s62) format for ease of transport through html. String62 format is a simple conversion of the values of the matrix into html legal ascii characters.

The encoded matrix width (x axis) is 26 characters, and comprise the consensus characters, the probabilities of each amino acid in the order A,B,C,D,E,F,G,H,I,K,L,M,N, P,Q,R,S,T,V,W,X,Y,Z (where B represents D and N, and Z represents Q and E, and X represents any amino acid), gap creation value, and gap extension value.

The length (y axis) of the matrix corresponds to the length of the sequences identified by PSI-BLAST. The order of the amino acids corresponds to the conserved amino acid sequence of the sequences identified using PSI-BLAST, with the N-terminal end at the top of the matrix. The probabilities of other amino acids at that position are represented for each amino acid along the x axis, below the respective single letter amino acid abbreviation.

Thus, each row of the profile consists of the highest scoring (consensus) amino acid, followed by the scores for each possible amino acid at that position in sequence matrix, the score for opening a gap that that position, and the score for continuing a gap at that position.

The string62 file is converted back into a profile for use in subsequent searches. The gap open field is set to 11 and the gap extension field is set to 1 along the x axis. The gap creation and gap extension values are known, based on the settings given to the PSI-BLAST algorithm. The matrix is exported to the standard GCG profile form. This format can be read by GenWeb.

The algorithm used to convert the string62 formatted file to the matrix is outlined in Table 1.

Table 1

- 1. if encoded character z then the value is blast score min
- 2. if encoded character Z then the value is blast score max
- 3. else if the encoded character is uppercase then its value is (64-(ascii # of char))
- 4. else if the encoded character is a digit the value is ((ascii # of char)-48)
- 5. else if the encoded character is not uppercase then the value is ((ascii # of char) 87)
- 6. ALL B positions are set to min of D and N amino acids at that row in sequence matrix
- 7. ALL Z positions are set to min of Q amd E amino acids at that row in sequence matrix
- 8. ALL X positions are set to min of all amino acids at that row in sequence matrix
- 9. kBLAST_SCORE_MAX=999;
- 10. kBLAST_SCORE_MIN=-999;
- 11. all gap opens are set to 11
- 12. all gap lens are set to 1

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Example 4: Identification of Novel Acyltransferase Related Amino Acid Sequences

The profile (Figure 1) is used in further queries to identify a number of previously unidentified proteins from yeast as novel acyltransferases. A protein is identified from an *Arabidopsis* protein sequence database (ATAT1) (SEQ ID NO:2). Sequences are also identified from nucleic acid databases (Table 2)

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Table 2

Database ID Number	BLAST Search Hits	Log probability
Saccharomyces cerevisiae		
gi 1078509	Limnanthes putative LPAAT	e-10 (SEQ ID
NO:217)		
gi 586485	Limnanthes putative LPAAT	e-13 (SEQ ID
NO:218)		

gi 320748	Limnanthes putative LPAAT	e-19 (SEQ ID
NO:219)		
gi 2506920	SUPPRESSES CTR1 (choline transp	oort mutant) (SEQ ID NO:220)
gi 549627	similar to CTR1	e-118 (SEQ ID
NO:221)		
gi 2133031	unidentified	(SEQ ID
NO:222)		
gi 2132939	unidentified	(SEQ ID
NO:223)		
gi 2132299	TAFAZZIN	e-14 (SEQ ID
NO:224)		

In Table 2, the gi number is the database identifier, the middle column shows the results of BLAST searches against the NCBI NR protein database, and the log probability number shows represents the log of the probability of such a match occurring by random chance. These proteins, including the ATAT1 protein sequence, are identified using the original PSI-BLAST search of the NCBI NR protein database. Thus, these proteins are novel acyltransferase related proteins with unidentified activities.

The Arabidopsis acyltransferase sequence, herein referred to as ATAT1, is also identified using the original PSI-BLAST search of the NCBI NR protein database, and did not have an annotated function.

Additional *Arabidopsis* amino acid sequences related to acyltransferases are identified from the databases, referred to as ATAT2est, ATAT3est, ATAT4est, ATAT5est, ATAT6est, ATAT7est, ATAT8est, ATAT9, ATAT10, and ATAT11est. Furthermore, *Arabidopsis* amino acid sequences are identified which demonstrate sequence similarity to known lysophosphatidic acid, referred to as ATLPAAT1. The sequences of ATAT9 and ATAT10 are identified from the database as genomic sequences, all other *Arabidopsis* sequences are identified as ESTs.

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Example 5: Sequence Analysis of the Novel Acyltransferases

To obtain the entire coding region corresponding to the *Arabidopsis* acyltransferase sequences, synthetic oligo-nucleotide primers are designed to amplify the 5' and 3' ends of partial cDNA clones containing acyltransferase related sequences. Primers are designed according to the respective *Arabidopsis* acyltransferase related sequences (Table 3) and used in Rapid Amplification of cDNA Ends (RACE) reactions (Frohman *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:8998-9002) using the Marathon cDNA amplification kit (Clontech Laboratories Inc, Palo Alto, CA). Primers with an R designation are used for 5' RACE reactions, and primers with an F designation are used for 3' RACE reactions.

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Table 3

	ATAT2		,
	ATAT2R1	CCATCCGCTTCAAGGGAACGACACCCATCA	(SEQ ID NO:135)
	ATAT2R2	TCCCTGTCTTGCTTGATGAACTTAAAGCTTG	(SEQ ID NO:136)
5	ATAT2R3	ACAGCAGGAGTGTCTGATGATGGCAGATTC	(SEQ ID NO:137)
		•	
	ATAT3		
	ATAT3R1	ACTGGAGTTCCAGCCAAAAATGCACCTGTC	C (SEQ ID NO:138)
	ATAT3R2	GATACACCCTTGAAATCAGGCGATTTTGCT	(SEQ ID NO:139)
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	ATAT4		
	ATAT4R1	TTGCAAATTCAATTCCTGTTTCACCGGGCC	(SEQ ID NO:140)
	ATAT4R2	GTTTTCTGCTATTCCAGAAGGCGTCAACAA	(SEQ ID NO:141)
15	ATAT5	;	
	ATAT5R1	CATTGAAGATCCGTCCGTGAAGTTNCCTTACC	C (SEQ ID NO:142)
	ATAT5R2	TCGAGCTGTGATCGATGATTGGCTGTGAAG	(SEQ ID NO:143)
	ATAT5F1	GTCTCTTCAAAAACACACACACACGTCTCT	(SEQ ID NO:144)
	ATAT5F2	GTCTCTTCAAAAACACACACACACGTCTCT	(SEQ ID NO:145)
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	ATAT6		
	H76348-F1	GTAGAGAGCCTTACTTGCTTCGGTTTAGTC	(SEQ ID NO:146)
	H76348-F2	ACGTCATCGTACCTGTTGCTATTGACTCAC (SEQ ID NO:147)
	H76348-R1	ACTTTTCCATTGTCAGGGACTCCTCGACAC	(SEQ ID NO:148)
25	H76348-R2	ACGGTGTAGGAAGGGAAAGGATTCAAAAGG	(SEQ ID NO:149)
	ATAT7		
	ATTS0193-	F1 GCGATGAACTACAGAGTCGGATTCTTCCTC	C (SEQ ID NO:150)
	ATTS0193-	F2 CCGGT ['] TTACGAGATTACGTTCTTGAACCAG	(SEQ ID NO:151)
30	ATTS0193-	R1 CAATGGAGACAAGGCTCGAAAGTGCTAAC	CC (SEQ ID NO:152)
	ATTS0193-	R2 ATTCTCTGAACATAGTTCGCCACGGTCATG	(SEQ ID NO:153)
	1		

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ATAT8

AA042618-F1 GAAATCCAACGCCTTCCCAATATCACTCTG (SEQ ID NO:154)

AA042618-F2 CTTCAACTTTCCATCAGGATCTTGGCACGT (SEQ ID NO:155)

AA042618-R1 ACCACTTGTTAGAGACCTTACCTGCTTAGG (SEQ ID NO:156)

AA042618-R2 TCCTACCTACACCATCCAATTTCTCGACCC (SEQ ID NO:157)

ATAT11

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ATAT11R1 CTGCGTCAAGTGAGCAACTCAGTTCTTGCA (SEQ ID NO:158)

ATAT11R2 TGGGAAGCAGCACGTTGTTCAGTATCGGAA (SEQ ID NO:159)

ATAT11R3 TAGCCTCTGTGTAATCTGTGCCCTCGGGGA (SEQ ID NO:160)

From the nucleic acid sequences obtained from the RACE reactions, protein sequence is predicted for each nucleic acid sequence using Macvector software. Nucleic acid sequences are provided for ATAT1 (SEQ ID NO:1), ATAT2 (SEQ ID NO:3), ATAT3 (SEQ ID NO:5), ATAT4 (SEQ ID NO:7), ATAT5 (SEQ ID NO:9), ATAT6 (SEQ ID NO:10), ATAT7 (SEQ ID NO:12), ATAT8 (SEQ ID NO:14), ATAT9 (SEQ ID NO:16), ATAT10 (SEQ ID NO:18), ATAT11 (SEQ ID NO:20) and ATLPAAT1 (SEQ ID NO:22), respectively.

The protein sequence derived from the ATAT1 (SEQ ID NO:2) nucleic acid sequence from Arabidopsis has a predicted molecular mass of 32.5 kDa, and a PI of 9.74. Alignment of the Arabidopsis acyltransferase with several LPAAT and G3PAAT shows that some of the domains that are conserved between LPAAT and G3PAAT are conserved in the new acyltransferase protein.

The ATAT2 nucleic acid sequence is predicted to encode a 312 amino acid protein (SEQ ID NO:4), with a molecular weight of 34.6 kD, and a pI of 9.99. The ATAT2 protein may also contain 2 to 3 transmembrane domains. However, the protein encoded by the ATAT2 nucleic acid sequence may be longer than predicted because of the absence of an inframe stop codon upstream of the ATG start codon used.

The ATAT3 nucleic acid sequence is predicted to encode a 398 amino acid protein (SEQ ID NO:6), with a molecular weight of 44.7 kD, and a pI of 5.62. The ATAT3 protein may contain 1 to 4 transmembrane domains. The ATAT4 nucleic acid sequence is predicted to encode a 317 amino acid protein (SEQ ID NO:8), with a molecular weight of 36.5 kD, and a pI of 9.67. The ATAT4 protein is predicted to have 2 to 5 transmembrane domains.

The ATLPAAT1 nucleic acid sequence is predicted to encode a 389 amino acid protein (SEQ ID NO:23), with a molecular weight of 43.7 kD, and a pI of 9.52. The ATLPAAT1 protein is predicted to have up to 3 transmembrane domains. The protein predicted from the ATLPAAT1 nucleic acid sequence is similar to LPAATs reported for *Brassica*, maize, and meadowfoam (described in PCT Publication WO 94/13814). The ATAT11 nucleic acid sequence is predicted to encode a 375 amino acid protein (SEQ ID NO:21), with a molecular weight of 43.5 kD, and a pI of 9.45. The deduced amino acid sequences of ATAT6 (SEQ ID NO:11), ATAT7 (SEQ ID NO:13), ATAT8 (SEQ ID NO:15), ATAT9 (SEQ ID NO:17), and ATAT10 (SEQ ID NO:19) are also provided

A sequence region approximately 30 amino acids upstream through approximately 100 amino acids downstream of the conserved amino acid sequences HXXXXD (Heath and Rock, (1998) *J. Bacteriol.* 180(6):1425-1430) and PEG (Neuwald (1997) *Curr Biol* 7:R465-R466) of the predicted amino acid sequences derived from the nucleic acid sequences of ATAT1, ATAT2, ATAT3, ATAT4, ATAT6, ATAT7, ATAT8, ATAT9, ATAT10, ATLPAAT1, and ATAT11 are compared to the amino acid sequences of lysophosphatidic acid acyltransferase (Jojoba AT (SEQ ID NO:162, the nucleic acid sequence is provided in SEQ ID NO:161), maize AT (PCT Publication WO 94/13814), PLSC coco(GenBank accession 1098605), PLSC Lim(GenBank accession 1209507), PLSC, Ecoli (GenBank accession 1209507), and PLSC Yeast(GenBank accession 464422)) and glycerol-3-phosphate acyltransferase (PLSB Ecoli(GenBank accession 130326) and PLSB Mouse(GenBank accession 2498786)) (Figure 2), and similarities are identified (Figure 2 and Figure 3).

Sequence comparisons reveal several classes of acyltransferases exist based on conserved amino acid sequences identified in the comparisons in Figure 2. For example, ATAT1, ATAT6, ATAT7, ATAT8, and ATAT9, contain the conserved amino acid sequences of VTYSXS(SEQ ID NO: 128), VXLTRXR(SEQ ID NO: 129), LXXGDLV(SEQ ID NO: 132) between the HXXXXXD and PEG sequences. In addition, ATAT1, ATAT6, ATAT7, ATAT8, and ATAT9 also contain the conserved sequences CPEGT(SEQ ID NO: 130) which comprises the PEG sequence, as well as IVPVA(SEQ ID NO: 131) and VANXXQ (SEQ ID NO: 134)(Figure 2) downstream of the PEG sequence. The sequences corresponding to ATAT1, ATAT7, and ATAT9 are the most closely related in this class, with similarities between ATAT1 and ATAT9 of 67.0%, between ATAT1 and ATAT7 of 58.2% and between ATAT9 and ATAT7 of 63.9% (Figure 3B).

Sequence comparisons also demonstrate that the sequence of ATLPAAT1 is most closely related to the jojoba LPAAT (82.3% similar), and maize (78.0% similar).

Furthermore, sequence analysis demonstrates that ATAT4 is the most divergent sequence with the highest similarity to ATAT10 (18.5%). The highest similarity (15.3%) to a known sequence is with a meadowfoam (*Limnanthes douglassi*) LPAAT. However, the sequences of ATAT4 and ATAT10 share several conserved peptide sequences with the amino acid sequences of ATAT2 and ATAT3 (Figure 2), VXNHXS (SEQ ID NO: 127) where the H comprises the conserved H of the HXXXXD sequence and FXXGAF (SEQ ID NO: 133) downstream of the PEG sequence.

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Example 6: Identification of Additional Acyltransferase Sequences

The novel *Arabidopsis* sequences identified above are used to search proprietary databases containing soybean and corn EST sequences. The results of this search identifies EST sequences from soybean (SEQ ID NO:24 through SEQ ID NO: 85) as well as from corn (SEQ ID NO: 86 through SEQ ID NO:126) as encoding acyltransferase related proteins.

Sequence comparisons between the various EST sequences and the complete Arabidopsis sequences reveals that the identified EST sequences demonstrate higher similarity to the various Arabidopsis sequences as determined by BLAST scores.

Expressed Sequence Tag (EST) sequences from soybean and corn databases are identified which are most closely related by BLAST score to ATAT1 (SEQ ID NOS:24-29 and SEQ ID NOS:86-88, respectively), ATAT2 (SEQ ID NO: 30 and SEQ ID NO:89, respectively), ATAT3 (SEQ ID NOS:31-35 and SEQ ID NOS:90-94, respectively), ATAT4 (SEQ ID NOS:36-44 and SEQ ID NOS:95-100, respectively), ATAT6 (SEQ ID NOS:45-49 and SEQ ID NO:101, respectively), ATAT7 (SEQ ID NOS:50-54 and SEQ ID NOS:102-103, respectively), ATAT8 (SEQ ID NOS:55-56 and SEQ ID NO:104, respectively), ATAT9 (SEQ ID NOS:57-79 and SEQ ID NOS:105-111, respectively), ATAT10 (SEQ ID NOS:80-81 and SEQ ID NO:112, respectively), ATAT11, (SEQ ID NOS:82-85 and SEQ ID NOS:123-126, respectively), and ATLPAAT1 (SEQ ID NOS: 113-122 respectively).

Example 7: Expression Construct Preparation

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A series of synthetic oligo nucleotide primers were prepared for use in Polymerase Chain Reactions (PCR) to amplify the entire DNA sequences encoding the various acyltransferase sequences identified above. The sequences are listed in Table 3.

Table 3

Primer	Sequence (listed 5'-3')	SEQ ID
		NO:
ATAT1F	AAGCTTGCATGCGTCGACACAATGGTTCATGCGACCAAGT	163
	CAG	
ATAT1R	GGTACCGTCGACTCACTTCTTGGTGTTGTTGATAG	164
ATAT2F	GGATCCGCGGCCGCACAATGACGAGCTTTACTACTTCCCT	165
	TCAT	
ATAT2R	GGATCCCCTGCAGGTTAGAGATCCATTGATTCTGCAAT	166
ATAT3F	GGATCCGCGGCCGCATAATGGAATCAGAGCTCAAAGAT	167
ATAT3R	GGATCCCCTGCAGGTCATTCTTCTTTCTGATGGAAATC	168
ATAT4F	GGATCCGCGCCCCACAATGACTCGTTCACAAGATGTTTC	169
	A	
ATAT4R	GGATCCCCTGCAGGTCACTTCTCTTCCAATCTAGCCAG	170
ATAT6F	GGATCCGCGCCCCACAATGTCCGGTAATAAGATCTCGAC	171
	TCTTCA	
ATAT6R	GGATCCCCTGCAGGTTATTTTTTTTTTGACAACTCCGTTAT	172
	TACCGG	
ATAT7F	ATATCCGCGCCCACAATGGTTATGGAGCAAGCTGGAA	173
ATAT7R	GGATCCCCTGCAGGTCAATGGAGACAAGGCTCGAAAGT	174
ATAT8F	GGATCCGCGCCCACAATGTCCGCCAAGATTTCAATATT	175
	cc	
ATAT8R	GGATCCCCTGCAGGTTAATTTTTCTTAACTACTCCATT	176
ATAT9F	GGATCCGCGCCCACAATGGGAGCTCAGGAGAAACGGCG	177
	CC	
ATAT9R	GGATCCCCTGCAGGTCACGTCTTCTCCTTCTTCACCGG	178
ATAT10F	GGATCCGCGCCCACAATGGCGGATCCTGATCTGTCTTC	179
	TCCT	
ATAT10R	GGATCCCCTGCAGGTTATGTTGGGGCCAAGTCAGGTGCAA	180
	AGAT	i
ATAT11F	GGATCCGCGGCCGAAAATGGAAAAAAAGAGTGTACCAAA	181

	TTCT	
ATAT11R	GGATCCCCTGCAGGTTATTTGTTTACTAATTTGAGGGAAT TTTTTG	182
ATLPAAT	TCGACCTGCAGGAAGCTTAAGGATGGTGATTGCTGC	183
1F		•
ATLPAAT	GGATCCGCGGCCGCTTACTTCTCCTTCTCCG	184
1R		
YSCAT1F	GGATCCGCGCCCACAATGTCTTTTAGGGATGTCCTAG	185
YSCAT1R	GGATCCCCTGCAGGTCAATCATCCTTACCCTTTGGTTTAC	186
	С	
YSCAT 1	ATGTCTTTTAGGGATGTCCTAGAAAGAGGAGATGAATTTT	187
KO F	CTGTGCGGTATTTCACACCG	
YSCAT 1	TCAATCATCCTTACCCTTTGGTTTACCCTCTGGAGGCAGA	188
KO R	AGATTGTACTGAGAGTGCAC	
YSCAT2F	GGATCCGCGCCCCACAATGAAGCATTCCCAAAAATACCG	189
	TAGG	
YSCAT2R	GGATCCCCTGCAGGTCAATGATTTTTTTTTCATCACAAATA	190
	C	
YSCAT 2	ATGAAGCATTCCCAAAAATACCGTAGGTATGGAATTTATG	191
KO F	CTGTGCGGTATTTCACACCG	
YSCAT 2	TCAATGATTTTTTTTCATCACAAATACAAGAATAAGAAAA	192
KO R	AGATTGTACTGAGAGTGCAC	
YSCAT	GGATCCGCGCCCCACAATGGGTTTTGTTGATTTCTTCGA	193
3F	AAC	
YSCAT	GGATCCCCTGCAGGTTATTTGGTCTCAATTTTAATATTTT	194
3R	TTTGC	
YSCAT 3	ATGGGTTTTGTTGATTTCTTCGAAACATATATGGTCGGTT	195
KO F	CTGTGCGGTATTTCACACCG	
YSCAT 3	TTATTTGGTCTCAATTTTAATATTTTTTTTGCAAGGACTCG	196
KO R	AGATTGTACTGAGAGTGCAC	
YSCAT	GGATCCGCGGCCGCACAATGGAAAAGTACACCAATTGGAG	197
4F	AGAC	
YSCAT	GGATCCCCTGCAGGCTACTTCCTCTTTTTACGTTGATCGC	198
4R	TG	
YSCAT 4	ATGGAAAAGTACACCAATTGGAGAGACAATGGTACGGGAA	199
KO F	CTGTGCGGTATTTCACACCG	
YSCAT 4	CTACTTCCTCTTTTTACGTTGATCGCTGATATATTCCTTC	200
KO R	AGATTGTACTGAGAGTGCAC	·

YSCAT	GGATCCGCGCCCACAATGCCTGCACCAAAACTCACGGA	201
5F	G	
YSCAT	GGATCCCCTGCAGGCTACGCATCTCCTTCTTTCCCTTC	. 202
5R		
YSCAT 5	ATGCCTGCACCAAAACTCACGGAGAAATCTGCCTCTTCCA	203
KO F	CTGTGCGGTATTTCACACCG	
YSCAT 5	CTACGCATCTCCTTCTTTCCCTTCTTCTTCTTCTTCTTCT	204
KO R	AGATTGTACTGAGAGTGCAC	
YSCAT	GGATCCGCGCCCCACAATGTCTGCTCCCGCTGCCGATCA	205
6F	TAACGC	
YSCAT	GGATCCCCTGCAGGTCATTCTTTCTTTTCGTGTTCTCTTT	206
6R	TCTG	•
YSCAT 6	ATGTCTGCTCCCGCTGCCGATCATAACGCTGCCAAACCTA	207
KO F	CTGTGCGGTATTTCACACCG	
YSCAT 6	TCATTCTTTTCGTGTTTCTCTTTTCTGTCTTACCAGC	208 .
KO R	AGATTGTACTGAGAGTGCAC	
YSCAT	GGATCCGCGCCCCACAATGCTGCATCAAAAAATAGCTCA	209
7F	TAAAGTTCG	
YSCAT	GGATCCCCTGCAGGTCAAAAAATAAAACAATAAAGTTTAT	210
7R	AAACTAACC	
YSCAT 7	ATGCTGCATCAAAAATAGCTCATAAAGTTCGAAAAGTCG	211
KO F	CTGTGCGGTATTTCACACCG	
YSCAT 7	TCAAAAATAAACAATAAAGTTTATAAACTAACCAAATT	212
KO R	AGATTGTACTGAGAGTGCAC	
YSCAT	GGATCCGCGGCCGCACAATGAGTGTGATAGGTAGGTTCTT	213
8F	G	
YSCAT	GGATCCCCTGCAGGTTAATGCATCTTTTTTACAGATGAAC	214
8R	C	
YSCAT 8	ATGAGTGTGATAGGTAGGTTCTTGTATTACTTGAGGTCCG	215
KO F	CTGTGCGGTATTTCACACCG	
YSCAT 8	TTAATGCATCTTTTTTACAGATGAACCTTCGTTATGGGTA	216
KO R	AGATTGTACTGAGAGTGCAC	

The entire coding regions for each of the acyltransferase sequences were amplified using the respective primers listed in the Table 3 above, cloned into the vector pCR2.1Topo (Invitrogen) or pZero (Invitrogen), and labeled as pCGN8558 (ATAT1), pCGN8564

(ATAT2), pCGB8565 (ATAT3), pCGN8566 (ATAT4), pCGN8918 (ATAT6), pCGN8913 (ATAT7), pCGN8904 (ATAT8), pCGN9970 (ATAT9), pCGN9940 (ATAT10), pCGN8567 (ATAT11), pCGN8632 (ATLPAAT1), pCGN9901 (YSCAT1 also referred to as gi2132299), pCGN9902 (YSCAT2, also referred to as gi1078509), pCGN9903 (YSCAT3, also referred to as gi2132939), pCGN9904 (YSCAT4, also referred to gi2133031), pCGN9905 (YSCAT5, also referred to as gi320748), pCGN9906 (YSCAT6, also referred to as gi549627), pCGN9907 (YSCAT7, also referred to as gi586485), and pCGN9908 (YSCAT8, also referred to as gi464422). The nucleic acid sequences for the respective yeast acyltransferase are provided YSCAT1 (SEQ ID NO:225), YSCAT2 (SEQ ID NO:226), YSCAT3 (SEQ ID NO:227), YSCAT4 (SEQ ID NO:228), YSCAT5 (SEQ ID NO:232), YSCAT6 (SEQ ID NO:230), YSCAT7 (SEQ ID NO:231), and YSCAT8 (SEQ ID NO:232).

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7A. Baculovirus Expression Constructs

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Constructs are prepared to direct the expression of the *Arabidopsis* ATAT sequences in cultured insect cells. The entire coding regions of ATAT1, 2, 3, 4, 6, 7, 8, 9, 10, and 11 are cloned into the vector pFastBac1 (Gibco-BRL, Gaithersburg, MD) digested with *Not*I and *Pst*I. The respective coding sequences were cloned as *NotI/Sse*8387I fragments. Double stranded DNA sequence was obtained to verify that no errors were introduced by PCR amplification. The resulting plasmid were designated pCGN9723 (ATAT1), pCGN9724 (ATAT2), pCGN9725 (ATAT3), pCGN9726 (ATAT4), pCGN9727 (ATAT5), pCGN9728 (ATAT7), pCGN9729 (ATAT8), pCGN9991 (ATAT9) pCGN9730 (ATAT10), pCGN9731 (ATAT11).

7B. Plant Expression Construct Preparation

A plasmid containing the napin cassette derived from pCGN3223 (described in USPN 5,639,790, the entirety of which is incorporated herein by reference) was modified to make it more useful for cloning large DNA fragments containing multiple restriction sites, and to allow the cloning of multiple napin fusion genes into plant binary transformation vectors. An adapter comprised of the self annealed oligonucleotide of sequence CGCGATTTAAATGGCGCGCCCTGCAGGCGCCCTGCAGGCGCCCTGCAGGCGCCCATTTAA (SEQ ID NO:233) AT was ligated into the cloning vector pBC SK+ (Stratagene) after digestion with the restriction endonuclease BssHII to construct vector pCGN7765. Plamids pCGN3223 and pCGN7765 were digested with Not1 and ligated together. The resultant vector, pCGN7770, contains the pCGN7765 backbone with the napin seed specific expression cassette from pCGN3223.

The cloning cassette, pCGN7787, essentially the same regulatory elements as pCGN7770, with the exception of the napin regulatory regions of pCGN7770 have been replaced with the double CAMV 35S promoter and the tml polyadenylation and transcriptional termination region.

A binary vector for plant transformation, pCGN5139, was constructed from pCGN1558 (McBride and Summerfelt, (1990) Plant Molecular Biology, 14:269-276). The polylinker of pCGN1558 was replaced as a HindIII/Asp718 fragment with a polylinker containing unique restriction endonuclease sites, AscI, PacI, XbaI, SwaI, BamHI, and NotI. The Asp718 and HindIII restriction endonuclease sites are retained in pCGN5139.

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A series of turbo binary vectors are constructed to allow for the rapid cloning of DNA sequences into binary vectors containing transcriptional initiation regions (promoters) and transcriptional termination regions.

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The plasmid pCGN8618 was constructed by ligating oligonucleotides 5'TCGAGGATCCGCGGCCGCAAGCTTCCTGCAGG-3') (SEQ ID NO:234) and 5'TCGACCTGCAGGAAGCTTGCGGCCGCGGATCC-3') (SEQ ID NO:235) into Sall/Xholdigested pCGN7770. A fragment containing the napin promoter, polylinker and napin 3'
region was excised from pCGN8618 by digestion with Asp718I; the fragment was bluntended by filling in the 5' overhangs with Klenow fragment then ligated into pCGN5139 that
had been digested with Asp718I and HindIII and blunt-ended by filling in the 5' overhangs
with Klenow fragment. A plasmid containing the insert oriented so that the napin promoter
was closest to the blunted Asp718I site of pCGN5139 and the napin 3' was closest to the
blunted HindIII site was subjected to sequence analysis to confirm both the insert orientation
and the integrity of cloning junctions. The resulting plasmid was designated pCGN8622.

The plasmid pCGN8619 was constructed by ligating oligonucleotides 5'TCGACCTGCAGGAAGCTTGCGGCCGCGGATCC -3') (SEQ ID NO:236) and 5'TCGAGGATCCGCGGCCGCAAGCTTCCTGCAGG-3') (SEQ ID NO:237) into Sall/Xholdigested pCGN7770. A fragment containing the napin promoter, polylinker and napin 3' region was removed from pCGN8619 by digestion with Asp718I; the fragment was bluntended by filling in the 5' overhangs with Klenow fragment then ligated into pCGN5139 that had been digested with Asp718I and HindIII and blunt-ended by filling in the 5' overhangs with Klenow fragment. A plasmid containing the insert oriented so that the napin promoter was closest to the blunted Asp718I site of pCGN5139 and the napin 3' was closest to the blunted HindIII site was subjected to sequence analysis to confirm both the insert orientation and the integrity of cloning junctions. The resulting plasmid was designated pCGN8623.

The plasmid pCGN8620 was constructed by ligating oligonucleotides 5'TCGAGGATCCGCGGCCGCAAGCTTCCTGCAGGAGCT -3') (SEQ ID NO:238) and 5'CCTGCAGGAAGCTTGCGGCCGCGGATCC-3') (SEQ ID NO:239) into Sall/SacIdigested pCGN7787. A fragment containing the d35S promoter, polylinker and tml 3' region
was removed from pCGN8620 by complete digestion with Asp718I and partial digestion with
NotI. The fragment was blunt-ended by filling in the 5' overhangs with Klenow fragment
then ligated into pCGN5139 that had been digested with Asp718I and HindIII and bluntended by filling in the 5' overhangs with Klenow fragment. A plasmid containing the insert

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oriented so that the d35S promoter was closest to the blunted Asp718I site of pCGN5139 and the tml 3' was closest to the blunted HindIII site was subjected to sequence analysis to confirm both the insert orientation and the integrity of cloning junctions. The resulting plasmid was designated pCGN8624.

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The plasmid pCGN8621 was constructed by ligating oligonucleotides 5'TCGACCTGCAGGAAGCTTGCGGCCGCGGATCCAGCT -3') (SEQ ID NO:240) and 5'GGATCCGCGGCCGCAAGCTTCCTGCAGG-3') (SEQ ID NO:241) into Sall/SacIdigested pCGN7787. A fragment containing the d35S promoter, polylinker and tml 3' region
was removed from pCGN8621 by complete digestion with Asp718I and partial digestion with
NotI. The fragment was blunt-ended by filling in the 5' overhangs with Klenow fragment
then ligated into pCGN5139 that had been digested with Asp718I and HindIII and bluntended by filling in the 5' overhangs with Klenow fragment. A plasmid containing the insert
oriented so that the d35S promoter was closest to the blunted Asp718I site of pCGN5139 and
the tml 3' was closest to the blunted HindIII site was subjected to sequence analysis to
confirm both the insert orientation and the integrity of cloning junctions. The resulting
plasmid was designated pCGN8625.

The coding regions of the various acyltransferase sequences were cloned as NotI/Sse83871 fragments into pCGN8622, pCGN8623, pCGN8624, and pCGN8625, for expression in sense or antisense orientations from a tissue preferential promoter, napin, or the 35S promoter. Fragments which were cloned into the pCGN8622 vector created the constructs pCGN8901 (ATAT1), pCGN8571 (ATAT2), pCGN8909 (ATAT3), pCGN8596 (ATAT4), pCGN8919 (ATAT6), pCGN8914 (ATAT7), pCGN8905 (ATAT8), pCGN9973 (ATAT9), pCGN9942 (ATAT10), pCGN8575 (ATAT11), and pCGN8633 (ATLPAAT1) for the sense expression of the respective coding sequences from the napin promoter. Fragments which were cloned into the pCGN8623 vector created the constructs pCGN8900 (ATAT1), pCGN8572 (ATAT2), pCGN8910 (ATAT3), pCGN8597 (ATAT4), pCGN8920 (ATAT6), pCGN8915 (ATAT7), pCGN8906 (ATAT8), pCGN9972 (ATAT9), pCGN9943 (ATAT10), pCGN8576 (ATAT11), and pCGN8634 (ATLPAAT1) for the antisense expression of the respective coding sequences from the napin promoter. Fragments which were cloned into the pCGN8624 vector created the constructs pCGN8903 (ATAT1), pCGN8573 (ATAT2), pCGN8911 (ATAT3), pCGN8598 (ATAT4), pCGN8921 (ATAT6), pCGN8916 (ATAT7), pCGN8907 (ATAT8), pCGN9971 (ATAT9), pCGN9944 (ATAT10), pCGN8577 (ATAT11), and pCGN8635 (ATLPAAT1) for the sense expression of the respective coding sequences

from the 35S promoter. Fragments which were cloned into the pCGN8625 vector created the constructs pCGN8902 (ATAT1) and pCGN9974 (ATAT9) for the antisense expression of the respective coding sequences from the 35S promoter.

In addition, the yeast acyltransferase coding sequences were cloned into the vector pCGN8624 creating the constructs pCGN9926 (YSCAT1), pCGN9927 (YSCAT2), pCGN9928 (YSCAT3), pCGN9929 (YSCAT4), pCGN9930 (YSCAT5), pCGN9931 (YSCAT6), pCGN9932 (YSCAT7), and pCGN9933 (YSCAT8). These constructs allow for the sense expression of the respective acyltransferase coding sequences from the 35S promoter in plant cells.

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Example 8: Plant Transformation

A variety of methods have been developed to insert a DNA sequence of interest into the genome of a plant host to obtain the transcription or transcription and translation of the sequence to effect phenotypic changes.

Transgenic Brassica plants are obtained by Agrobacterium-mediated transformation as described by Radke et al. (Theor. Appl. Genet. (1988) 75:685-694; Plant Cell Reports (1992) 11:499-505). Transgenic Arabidopsis thaliana plants may be obtained by Agrobacterium-mediated transformation as described by Valverkens et al., (Proc. Nat. Acad. Sci. (1988) 85:5536-5540), or as described by Bent et al. ((1994), Science 265:1856-1860), or Bechtold et al. ((1993), C.R.Acad.Sci, Life Sciences 316:1194-1199) or Clough, et al. (1998) Plant J., 16:735-43. Other plant species may be similarly transformed using related techniques.

Alternatively, microprojectile bombardment methods, such as described by Klein *et al.* (*Bio/Technology 10*:286-291) may also be used to obtain nuclear transformed plants.

The above results demonstrate that the nucleic acid sequences identified encode proteins which are related to protein sequences encoding acyltransferase proteins. Such acyltransferase sequences find use in preparing expression constructs for plant transformations.

All publications and patent applications mentioned in this specification are indicative of the level of skill of those skilled in the art to which this invention pertains. All

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publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the appended claim.

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Claims

What is Claimed is:

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An isolated DNA sequence encoding an enzyme of the class of acyltransferase-like
 proteins,

wherein said enzyme includes the amino acid sequence of SEQ ID NO: 127 (VxNHxS) wherein the H is the conserved Histidine residue in the conserved peptide sequence HXXXXD of said acyltransferase-like protein, x representing any amino acid.

2. An isolated DNA sequence encoding an enzyme of the class of acyltransferase-like proteins,

wherein said enzyme includes the amino acid sequence of SEQ ID NO: 128 (VTYSxS) within about 30 amino acids downstream from the conserved amino acid sequence HXXXXD of said acyltransferase-like protein, x representing any amino acid.

3. An isolated DNA sequence encoding an enzyme of the class of acyltransferase-like proteins,

wherein said enzyme includes the amino acid sequence of SEQ ID NO: 129 (VxLTRxR) within about 60 amino acids downstream from the conserved amino acid sequence HXXXXD of said acyltransferase-like protein, x representing any amino acid.

4. An isolated DNA sequence encoding an enzyme of the class of acyltransferase-like proteins,

wherein said enzyme includes the amino acid sequence of SEQ ID NO: 132 (LxxGDLV) within about 20 amino acids upstream of the conserved amino acid sequence PEG of said acyltransferase-like protein, x representing any amino acid.

- 5. An isolated DNA sequence encoding an enzyme of the class of acyltransferase-like proteins,
- wherein said enzyme includes the amino acid sequence of SEQ ID NO: 130 (CPEGT) containing the conserved amino acid sequence PEG of said acyltransferase-like protein.

6. An isolated DNA sequence encoding an enzyme of the class of acyltransferase-like proteins,

wherein said enzyme includes the amino acid sequence of SEQ ID NO: 133 (FxxGAF) within about 20 amino acids downstream from the conserved amino acid sequence PEG of said acyltransferase-like protein, x representing any amino acid.

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7. An isolated DNA sequence encoding an enzyme of the class of acyltransferase-like proteins,

wherein said enzyme includes the amino acid sequence of SEQ ID NO: 131 (IVPVA) within about 40 amino acids downstream from the conserved amino acid sequence PEG of said acyltransferase-like protein.

8. An isolated DNA sequence encoding an enzyme of the class of acyltransferase-like proteins,

wherein said enzyme includes the amino acid sequence of SEQ ID NO: 134 (VANxxQ) within about 110 amino acids downstream from the conserved amino acid sequence PEG of said acyltransferase-like protein, x representing any amino acid.

- 9. A DNA sequence encoding an enzyme of the class of acyltransferase-like proteins, said DNA sequence obtainable by the steps comprising:
 - (a) using the profile of Figure 1 to search a nucleic acid sequence database;
 - (b) obtaining a probability score for nucleic acid sequences in said sequence database using the Smith-Waterman algorithm; and
 - (c) selecting a nucleic acid sequence having a probability score of less than about 1.
 - 10. The DNA encoding sequence according to Claim 9, wherein said DNA sequence is an encoding sequence.
- 30 11. The DNA encoding sequence according to Claim 9, wherein said DNA sequence is an EST.

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12. The DNA encoding sequence according to any one of Claims 1 to 11, wherein said acyltransferase-like protein is from a plant.

- 13. A construct comprising a DNA sequence of any one of Claims 1 to 11 linked to a
 heterologous transcriptional and translational initiation region functional in a host cell.
 - 14. The construct according to Claim 13 wherein said host cell is a plant cell.
 - 15. A plant cell comprising a DNA construct according to Claim 13.
 - 16. A plant comprising a cell according to Claim 15.

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- 17. The DNA encoding sequence of any one of 1 to 11 wherein said acyltransferaselike protein is from Arabidopsis thaliana.
 - 18. The DNA encoding sequence of any one of 1 to 11 wherein said acyltransferaselike protein is from corn.
- 20 19. The DNA encoding sequence of Claim 18 wherein said sequence comprises and EST selected from the group consisting of SEQ ID NO: 86 through SEQ ID NO: 126.
 - 20. The DNA encoding sequence of any one of 1 to 11 wherein said acyltransferase-like protein is from soybean.
 - 21. The DNA encoding sequence of Claim 20 wherein said sequence comprises and EST selected from the group consisting of SEQ ID NO: 24 through SEQ ID NO: 85.
- The DNA encoding sequence of any one of Claims 2, 3, 4, 5, 7 and 8 wherein
 said acyltransferase-like protein is selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14 and SEQ ID NO: 16.

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23. The DNA encoding sequence of either of Claim 1 and Claim 6 wherein said acyltransferase-like protein is selected from the group consisting of SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7 and SEQ ID NO: 18.

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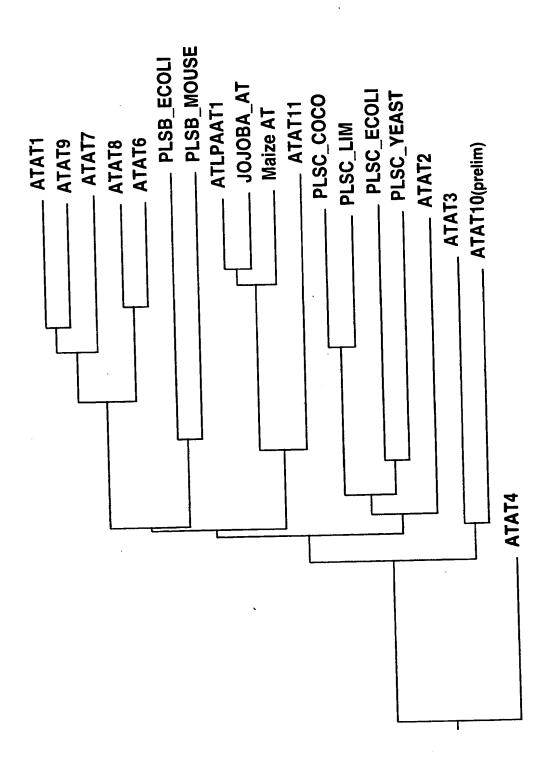


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	61	14.4	14.4	14.4		Q:7	15.9	10.6	<u>0</u>	14.4	12.0	5	2.71	12.9	12.9	15.3	12.3	13.8	17.6	17.2	18.5		6
	18	14.4	13.7	17.1		2	27	123	11.6	16.4	15.1	3	5.6	15.8	17.1	14.4	17.8	15.1	15.8	30.8		74.1	18
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1320

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Ala Ala Leu Lys Leu Lys Ile Phe Val Ala Thr Val Gly Leu Arg Glu 65 70 75 80

Pro Glu Ile Glu Ser Val Ala Arg Ala Val Leu Pro Lys Phe Tyr Met
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Asp Asp Val Ser Met Asp Thr Trp Arg Val Phe Ser Ser Cys Lys 100 105 110

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Lys Glu His Leu Arg Ala Asp Glu Val Ile Gly Thr Glu Leu Ile Val 130 135 140

Asn Arg Phe Gly Phe Val Thr Gly Leu Ile Arg Glu Thr Asp Val Asp 145 150 155 160

Gln Ser Ala Leu Asn Arg Val Ala Asn Leu Phe Val Gly Arg Arg Pro 165 170 175

Gln Leu Gly Leu Gly Lys Pro Ala Leu Thr Ala Ser Thr Asn Phe Leu 180 185 190

Ser Leu Cys Glu Glu His Ile His Ala Pro Ile Pro Glu Asn Tyr Asn 195 200 205

His Gly Asp Gln Gln Leu Gln Leu Arg Pro Leu Pro Val Ile Phe His 210 220

Asp Gly Arg Leu Val Lys Arg Pro Thr Pro Ala Thr Ala Leu Ile Ile 225 230 235 240

Leu Leu Trp Ile Pro Phe Gly Ile Ile Leu Ala Val Ile Arg Ile Phe 245 250 255

Leu Gly Ala Val Leu Pro Leu Trp Ala Thr Pro Tyr Val Ser Gln Ile 260 265 270

Phe Gly Gly His Ile Ile Val Lys Gly Lys Pro Pro Gln Pro Pro Ala 275 280 285

Ala Gly Lys Ser Gly Val Leu Phe Val Cys Thr His Arg Thr Leu Met

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Val Lys Val Met Val Met Val Ser Phe Phe Gly Ile Lys Lys Glu Gly 100 105 110

Phe Arg Ala Gly Arg Ala Val Leu Pro Lys Tyr Phe Leu Glu Asp Val 115 120 125

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Val Ser Asp Asp Leu Pro Gln Val Met Ile Glu Gly Phe Leu Arg Asp 145 150 155 160

Tyr Leu Glu Ile Asp Val Val Gly Arg Glu Met Lys Val Val Gly 165 170 175

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Lys Pro Ser Gln Arg Lys Gly Cys Leu Phe Val Cys Asn His Arg Thr
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Lys Thr Val Thr Tyr Ser Leu Ser Arg Val Ser Glu Ile Leu Ala Pro
Ile Lys Thr Val Arg Leu Thr Arg Asp Arg Val Ser Asp Gly Gln Ala
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Thr Thr Cys Arg Glu Pro Tyr Leu Leu Arg Phe Ser Pro Leu Phe Thr
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Phe Phe Tyr Gly Thr Thr Ala Ser Gly Leu Lys Ala Leu Asp Pro Leu
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Pro Val Ser Gly Ala Thr Cys Gln Asp Pro Asp Gly Lys Leu Lys Phe
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155

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Lys Asp Leu Pro Glu Ser Asp Asp Ala Ile Ala Gln Trp Cys Arg Asp 260 265 270

Gln Phe Val Ala Lys Asp Ala Leu Leu Asp Lys His Ile Ala Ala Asp 275 280 285

Thr Phe Pro Gly Gln Gln Glu Gln Asn Ile Gly Arg Pro Ile Lys Ser 290 295 300

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Phe Phe Ser Gly Leu Phe Ile Asn Phe Ile Gln Ala Ile Cys Phe Val
Leu Val Arg Pro Leu Ser Lys Thr Tyr Arg Arg Ile Asn Arg Val Leu
Val Glu Leu Leu Trp Leu Glu Leu Ile Trp Leu Val Asp Trp Trp Ala
Ser Val Lys Ile Lys Leu Phe Thr Asp Pro Asp Thr Phe Arg Leu Met
Gly Lys Glu His Ala Leu Val Ile Ser Asn His Arg Ser Asp Ile Asp
Trp Leu Val Gly Trp Val Leu Ala Gln Arg Ser Gly Cys Leu Gly Ser
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Thr Leu Ala Val Met Lys Lys Ser Ser Lys Phe Leu Pro Val Ile Gly 120 Trp Ser Met Trp Phe Ser Glu Tyr Leu Phe Leu Glu Arg Ser Trp Ala 135 Lys Asp Glu Ser Thr Leu Lys Leu Gly Leu Gln Arg Leu Lys Asp Tyr. 155 Pro Leu Pro Phe Trp Leu Ala Leu Phe Val Glu Gly Thr Arg Phe Thr Gln Ala Lys Leu Leu Ala Ala Gln Glu Tyr Ala Thr Ser Met Gly Leu Pro Val Pro Arg Asn Thr Leu Ile Pro Arg Thr Lys Gly Phe Val Ser 200 Ala Val Ser His Met Arg Ser Phe Val Pro Ala Ile Tyr Asp Val Thr Val Ala Ile Pro Lys Ser Ser Ser Gln Pro Thr Met Leu Arg Leu Phe Lys Gly Gln Pro Ser Thr Val His Val His Ile Lys Arg Arg Ser Met Lys Asp Leu Pro Glu Ala Ala Asp Asp Val Ala Gln Trp Cys Arg Asp 265 Thr Phe Val Ala Lys Asp Ala Leu Leu Asp Lys His Asn Val Asp Asp 280 Thr Phe Gly Asp Glu Tyr Leu Gln Asp Thr Gly Arg Pro Leu Lys Ser Leu Phe Val Ala Val Ser Trp Ala Leu Ile Leu Ile Leu Gly Gly Leu Lys Phe Leu Arg Trp Ser Ser Leu Leu Ser Ser Trp Lys Gly Val Ala 325 330 Phe Ser Ala Ala Cys Leu Val Leu Val Thr Ile Leu Met Gln Ile Leu Ile Gln Phe Ser Gln Ser Glu Arg Ser Thr Pro Ala Lys Val Ala Pro Gly Lys Pro Lys Asn Met Val Ser Glu Pro Thr Glu Thr Gln Arg His Lys Gln His

<210> 163

<211> 43

<212> DNA

<213> Artificial Sequence

<220>

385

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<210> 164

<211> 35

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	20> 23>	Description of Artificial Sequence:Synthetic Oligonucleotide	
		184 egegg eegettaett eteettetee g	31
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<211> 381 <212> PRT

<213> Saccharomyces sp.

<220>

<400> 217

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Tyr Pro Arg Arg Ser Pro Leu Trp Arg Phe Leu Ser Tyr Ser Thr Ser 20 25 30

Leu Leu Thr Phe Gly Val Ser Lys Leu Leu Phe Thr Cys Tyr Asn 35 40 45

Val Lys Leu Asn Gly Phe Glu Lys Leu Glu Thr Ala Leu Glu Arg Ser 50 55 60

Lys Arg Glu Asn Arg Gly Leu Met Thr Val Met Asn His Met Ser Met 65 70 75 80

Val Asp Asp Pro Leu Val Trp Ala Thr Leu Pro Tyr Lys Leu Phe Thr 85 90 95

Ser Leu Asp Asn Ile Arg Trp Ser Leu Gly Ala His Asn Ile Cys Phe 100 105 110

Gln Asn Lys Phe Leu Ala Asn Phe Phe Ser Leu Gly Gln Val Leu Ser 115 120 125

Thr Glu Arg Phe Gly Val Gly Pro Phe Gln Gly Ser Ile Asp Ala Ser 130 135 140

Ile Arg Leu Leu Ser Pro Asp Asp Thr Leu Asp Leu Glu Trp Thr Pro 145 150 155 160

His Ser Glu Val Ser Ser Ser Leu Lys Lys Ala Tyr Ser Pro Pro Ile 165 170 175

Ile Arg Ser Lys Pro Ser Trp Val His Val Tyr Pro Glu Gly Phe Val
180 185 190

Leu Gln Leu Tyr Pro Pro Phe Glu Asn Ser Met Arg Tyr Phe Lys Trp
195 200 205

Gly Ile Thr Arg Met Ile Leu Glu Ala Thr Lys Pro Pro Ile Val Val 210 215 220

Pro Ile Phe Ala Thr Gly Phe Glu Lys Ile Ala Ser Glu Ala Val Thr 225 230 235 240

Asp Ser Met Phe Arg Gln Ile Leu Pro Arg Asn Phe Gly Ser Glu Ile 245 250 255

Asn Val Thr Ile Gly Asp Pro Leu Asn Asp Asp Leu Ile Asp Arg Tyr 260 265 270

Arg Lys Glu Trp Thr His Leu Val Glu Lys Tyr Tyr Asp Pro Lys Asn 275 280 285

Pro Asn Asp Leu Ser Asp Glu Leu Lys Tyr Gly Lys Glu Ala Gln Asp 290 295 300

Leu Arg Ser Arg Leu Ala Ala Glu Leu Arg Ala His Val Ala Glu Ile

305 310 . 315 320 .

Arg Asn Glu Val Arg Lys Leu Pro Arg Glu Asp Pro Arg Phe Lys Ser 325 330 335

Pro Ser Trp Trp Lys Arg Phe Asn Thr Thr Glu Gly Lys Ser Asp Pro 340 345 350

Asp Val Lys Val Ile Gly Glu Asn Trp Ala Ile Arg Arg Met Gln Lys 355 360 365

Phe Leu Pro Pro Glu Gly Lys Pro Lys Gly Lys Asp Asp 370 375 380

<210> 218

<211> 396

<212> PRT

<213> Saccharomyces sp.

<220>

<400> 218

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Gly Asn Pro Phe Ile Lys Gly Leu Gln Arg Leu Leu Ile Ala Cys Leu 20 25 30

Phe Ile Ser Gly Ser Leu Ser Ile Val Val Phe Gln Ile Cys Leu Gln 35 40 45

Val Leu Leu Pro Trp Ser Lys Ile Arg Phe Gln Asn Gly Ile Asn Gln 50 60

Ser Lys Lys Ala Phe Ile Val Leu Cys Met Ile Leu Asn Met Val 65 70 75 80

Ala Pro Ser Ser Leu Asn Val Thr Phe Glu Thr Ser Arg Pro Leu Lys 85 90 95

Asn Ser Ser Asn Ala Lys Pro Cys Phe Arg Phe Lys Asp Arg Ala Ile 100 105 110

Ile Ile Ala Asn His Gln Met Tyr Ala Asp Trp Ile Tyr Leu Trp Trp 115 120 125

Leu Ser Phe Val Ser Asn Leu Gly Gly Asn Val Tyr Ile Ile Leu Lys 130 140

Lys Ala Leu Gln Tyr Ile Pro Leu Leu Gly Phe Gly Met Arg Asn Phe 145 150 155 160

Lys Phe Ile Phe Leu Ser Arg Asn Trp Gln Lys Asp Glu Lys Ala Leu 165 170 175

Thr Asn Ser Leu Val Ser Met Asp Leu Asn Ala Arg Cys Lys Gly Pro 180 185 190

Leu Thr Asn Tyr Lys Ser Cys Tyr Ser Lys Thr Asn Glu Ser Ile Ala 195 200 205

Ala Tyr Asn Leu Ile Met Phe Pro Glu Gly Thr Asn Leu Ser Leu Lys 210 220

Thr Arg Glu Lys Ser Glu Ala Phe Cys Gln Arg Ala His Leu Asp His 225 230 235 240

Val Gln Leu Arg His Leu Leu Pro His Ser Lys Gly Leu Lys Phe 245 250 255 Ala Val Glu Lys Leu Ala Pro Ser Leu Asp Ala Ile Tyr Asp Val Thr Ile Gly Tyr Ser Pro Ala Leu Ang Thr Glu Tyr Val Gly Thr Lys Phe 290 Lys Lys Ile Phe Leu Arg 280 Met Gly Val Tyr Pro Glu Lys Val Asp 300 Glu Lys Val Asp 310 Arg 295 Met Gly Val Tyr Pro Glu Lys Val Asp 320 Glu Val Phe Arg Glu Phe Arg Val Leu Gly Jajo Trp Leu Glu Asp 320 Glu Val Phe Re Arg Tyr Tyr Asn Thr Gly Jajo Trp Lys Glu Lys Asp 335 Gln Asn Asp Asp 340 Try Tyr Asn Thr Gly Gln Phe Lys Ser Asn Ala Lys 355 Glu Thr Leu Thr Pro Arg Ile Leu Ser Tyr Tyr Tyr Gly Phe Gln Ass 370 The Leu Leu Ile Leu Thr Pro Arg Ile Leu Leu Ser Tyr Tyr Tyr Gly Phe Phe Ala Sep Leu Ile Leu Val Phe Val Met Lys Lys Asn His

<210> 219

<211> 479

<212> PRT

<213> Saccharomyces sp.

<220>

Phe Tyr Thr Lys Trp Glu Phe Leu Gln Lys Leu Arg Lys Gly Glu Asp Leu Ala Glu Trp Pro Gln Leu Lys Phe Leu Gly Trp Gly Lys Met Phe 200 Asn Phe Pro Arg Leu Asp Leu Leu Lys Asn Ile Phe Phe Lys Asp Glu 210 215 220 Thr Leu Ala Leu Ser Ser Asn Glu Leu Arg Asp Ile Leu Glu Arg Gln Asn Asn Gln Ala Ile Thr Ile Phe Pro Glu Val Asn Ile Met Ser Leu Glu Leu Ser Ile Ile Gln Arg Lys Leu His Gln Asp Phe Pro Phe Val 260 265 Ile Asn Phe Tyr Asn Leu Leu Tyr Pro Arg Phe Lys Asn Phe Thr Thr 280 Leu Met Ala Ala Phe Ser Ser Ile Lys Asn Ile Lys Arg Lys Lys Asn Arg Asn Asn Ile Ile Lys Glu Ala Arg Tyr Leu Phe His Arg Glu Leu Asp Lys Leu Val His Lys Ser Met Lys Met Glu Ser Ser Lys Val Ser 330 Asp Lys Thr Thr Pro Pro Met Ile Val Asp Asn Ser Tyr Leu Leu Thr Lys Lys Glu Glu Ile Ser Ser Gly Lys Pro Lys Val Val Arg Ile Asn Pro Tyr Ile Tyr Asp Val Thr Ile Ile Tyr Tyr Arg Val Lys Tyr Thr 375 Asp Ser Gly His Asp His Thr Asn Gly Asp Leu Arg Leu His Lys Gly 390 395 Tyr Gln Leu Glu Gln Ile Ser Pro Thr Ile Phe Glu Met Ile Gln Pro Glu Met Glu Ser Glu Asn Asn Ile Lys Asp Lys Asp Pro Ile Val Val Met Val Asn Val Lys Lys His Gln Ile Gln Pro Leu Leu Ala Tyr Asn 435 440 Asp Glu Ser Leu Glu Lys Trp Leu Glu Asn Arg Trp Ile Glu Lys Asp 455 Arg Leu Ile Glu Ser Leu Gln Lys Asn Ile Lys Ile Glu Thr Lys 475

<210> 220

<400> 220

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Phe Leu Pro Asn Thr Ile Arg Lys Pro Ser Lys Val Met Thr Ala Cys 20 25 30

<211> 300

<212> PRT

<213> Saccharomyces sp.

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<210> 221

<211> 759

<212> PRT

<213> Saccharomyces sp.

<400> 221

Met Pro Ala Pro Lys Leu Thr Glu Lys Phe Ala Ser Ser Lys Ser Thr 1 5 10 15

Gln Lys Thr Thr Asn Tyr Ser Ser Ile Glu Ala Lys Ser Val Lys Thr 20 25 30

Ser Ala Asp Gln Ala Tyr Ile Tyr Gln Glu Pro Ser Ala Thr Lys Lys 35 40 45

Ile Leu Tyr Ser Ile Ala Thr Trp Leu Leu Tyr Asn Ile Phe His Cys

Phe Phe Arg Glu Ile Arg Gly Arg Gly Ser Phe Lys Val Pro Gln Gln Gly Pro Val Ile Phe Val Ala Ala Pro His Ala Asn Gln Phe Val Asp Pro Val Ile Leu Met Gly Glu Val Lys Lys Ser Val Asn Arg Arg Val 100 105 110Ser Phe Leu Ile Ala Glu Ser Ser Leu Lys Gln Pro Pro Ile Gly Phe Leu Ala Ser Phe Phe Met Ala Ile Gly Val Val Arg Pro Gln Asp Asn 135 Leu Lys Pro Ala Glu Gly Thr Ile Arg Val Asp Pro Thr Asp Tyr Lys Arg Val Ile Gly His Asp Thr His Phe Leu Thr Asp Cys Met Pro Lys Gly Leu Ile Gly Leu Pro Lys Ser Met Gly Phe Gly Glu Ile Gln Ser Ile Glu Ser Asp Thr Ser Leu Thr Leu Arg Lys Glu Phe Lys Met Ala 200 Lys Pro Glu Ile Lys Thr Ala Leu Leu Thr Gly Thr Thr Tyr Lys Tyr 215 Ala Ala Lys Val Asp Gln Ser Cys Val Tyr His Arg Val Phe Glu His Leu Ala His Asn Asn Cys Ile Gly Ile Phe Pro Glu Gly Gly Ser His Asp Arg Thr Asn Leu Leu Pro Leu Lys Ala Gly Val Ala Ile Met Ala 265 Leu Gly Cys Met Asp Lys His Pro Asp Val Asn Val Lys Ile Val Pro Cys Gly Met Asn Tyr Phe His Pro His Lys Phe Arg Ser Arg Ala Val 295 Val Glu Phe Gly Asp Pro Ile Glu Ile Pro Lys Glu Leu Val Ala Lys 310 Tyr His Asn Pro Glu Thr Asn Arg Asp Ala Val Lys Glu Leu Leu Asp 330 Thr Ile Ser Lys Gly Leu Gln Ser Val Thr Val Thr Cys Ser Asp Tyr 345 Glu Thr Leu Met Val Val Gln Thr Ile Arg Arg Leu Tyr Met Thr Gln Phe Ser Thr Lys Leu Pro Leu Pro Leu Ile Val Glu Met Asn Arg Arg Met Val Lys Gly Tyr Glu Phe Tyr Arg Asn Asp Pro Lys Ile Ala Asp 395 385 390 Leu Thr Lys Asp Ile Met Ala Tyr Asn Ala Ala Leu Arg His Tyr Asn 410 Leu Pro Asp His Leu Val Glu Glu Ala Lys Val Asn Phe Ala Lys Asn 425

Leu Gly Leu Val Phe Phe Arg Ser Ile Gly Leu Cys Ile Leu Phe Ser Leu Ala Met Pro Gly Ile Ile Met Phe Ser Pro Val Phe Ile Leu Ala Lys Arg Ile Ser Gln Glu Lys Ala Arg Thr Ala Leu Ser Lys Ser Thr 475 Val Lys Ile Lys Ala Asn Asp Val Ile Ala Thr Trp Lys Ile Leu Ile Gly Met Gly Phe Ala Pro Leu Leu Tyr Ile Phe Trp Ser Val Leu Ile Thr Tyr Tyr Leu Arg His Lys Pro Trp Asn Lys Ile Tyr Val Phe Ser Gly Ser Tyr Ile Ser Cys Val Ile Val Thr Tyr Ser Ala Leu Ile Val 530 540 Gly Asp Ile Gly Met Asp Gly Phe Lys Ser Leu Arg Pro Leu Val Leu Ser Leu Thr Ser Pro Lys Gly Leu Gln Lys Leu Gln Lys Asp Arg Arg Asn Leu Ala Glu Arg Ile Ile Glu Val Val Asn Asn Phe Gly Ser Glu Leu Phe Pro Asp Phe Asp Ser Ala Ala Leu Arg Glu Glu Phe Asp Val Ile Asp Glu Glu Glu Asp Arg Lys Thr Ser Glu Leu Asn Arg Arg Lys Met Leu Arg Lys Gln Lys Ile Lys Arg Gln Glu Lys Asp Ser Ser Ser Pro Ile Ile Ser Gln Arg Asp Asn His Asp Ala Tyr Glu His His Asn Gln Asp Ser Asp Gly Val Ser Leu Val Asn Ser Asp Asn Ser Leu Ser Asn Ile Pro Leu Phe Ser Ser Thr Phe His Arg Lys Ser Glu Ser 680 Ser Leu Ala Ser Thr Ser Val Ala Pro Ser Ser Ser Glu Phe Glu 695 Val Glu Asn Glu Ile Leu Glu Glu Lys Asn Gly Leu Ala Ser Lys Ile Ala Gln Ala Val Leu Asn Lys Arg Ile Gly Glu Asn Thr Ala Arg Glu Glu Gly Lys Glu Gly Asp Ala

<210> 222 <211> 743

<212> PRT

<213> Saccharomyces sp.

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شبعا أوكيز

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